AFRL-RH-BR-TR-2008-0062

WARFIGHTER SUSTAINABILITY: MAXIMIZING HUMAN PERFORMANCE IN HOSTILE ENVIRONMENTS

Brent C. Ruby
Dustin Slivka
John Cuddy
Walter Hailes

University of Montana

October 2008

Air Force Research Laboratory
Human Effectiveness Directorate
Biosciences and Protection Division
Biobehavioral Performance Branch
Brooks-City-Base, TX 78235

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MARK M. HOFFMAN
Deputy Division Chief
Biosciences and Protection Division

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Public Affairs Case File No. 09-091, 5 March 2009.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. REPORT DATES**
11-10- 2008	Final Technical Report	11 Aug 06 – 11 Sep 08

4. TITLE AND SUBTITLE

Warfighter Sustainability: Maximizing Human Performance in Hostile Environments

5a. CONTRACT NUMBER
FA8650-06-2-6740

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
62202F

6. AUTHOR(S)
Brent C. Ruby, Dustin Slivka, John Cuddy, Walter Hailes

5d. PROJECT NUMBER
7757

5e. TASK NUMBER
P9

5f. WORK UNIT NO.
15

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Montana
Montana Center for Work Physiology and Exercise Metabolism (WPEM)
32 Campus Drive
Missoula, MT, 59812

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Materiel Command
Air Force Research Laboratory
Human Effectiveness Directorate
Biosciences and Protection Division
Biobehavioral Performance Branch
2485 Gillingham Dr
Brooks City-Base, Texas, 78235-5105

10. SPONSOR/MONITOR'S ACRONYM(S)
711 HPW/RHPF

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-RH-BR-TR-2008-0062

12. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution A. Approved for public release. Distribution unlimited, Public Affairs Case File no. 09-091, 5 March 2009.

13. SUPPLEMENTARY NOTES

14. ABSTRACT: The goal of this effort was to improve training and sustainment programs for the Air Force Special Operations Command. Interventions are sought to enhance physical strength, endurance, fatigue resistance, and mental resilience. Over the course of this funding cycle, the effects of nutrition on human performance have been studied under various environmental conditions in the laboratory and austere environments. Similarly, the practical usefulness of biomarker detection during field environments has been evaluated. The overall purpose of this series of investigations has been to better understand strategies associated with training and nutritional interventions that optimize performance and enhance recovery from stressful conditions. In addition, the use of physiological monitoring devices for field data collection has been rigidly evaluated. Additional information has also been collected during events that better capture the human ceiling for energy expenditure and hydration demands. The purpose of these series of investigations was to pool research results from controlled laboratory study and field data collection efforts to provide information to the Air Force Special Operations Command in the areas of **Mission Preparedness and Recovery** and **Mission Performance**. These data clearly demonstrate that mission performance can be altered by a variety of environmental factors that impede performance. However, the environmental concerns are of less concern compared to factors that can be altered and/or controlled by the warfighter. These include training status, approaches to training and also adequately meeting the nutritional and hydration demands of the required muscle work.

15. SUBJECT TERMS
Fatigue; Injuries; Nutrition; Physical Training; Special Forces

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a.NAME OF RESPONSIBLE PERSON**
					2d Lt. Lynette Lennemann
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b.TELEPHONE NUMBER** *(include area code)*
Uncl	Uncl	Uncl	SAR	190	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std.

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PREFACE

This research was funded by a Program Element 62202F Congressional Add. The contractor was the University of Montana, under contract FA8650-06-2-6740. The contract and work unit (7757P915) were managed by the Biobehavioral Performance Branch (AFRl/RHPF) of the Air Force Human Research Laboratory at Brooks City-Base, TX.

Executive Summary

Background:

During the Fall of 2005, the University of Montana submitted proposals for Congressional funding consideration. These proposal efforts were the result of prior research collaborations with various branches of the military, primarily the Air Force Special Operations Command (AFSOC), and the US Forest Service. The University of Montana proposed a series of laboratory and field investigations that could provide mission specific data to AFSOC to improve approaches to pre-deployment training, mission performance, and muscle recovery from arduous and extended operation in hostile environments.

Additional discussions were initiated with key researchers, clinicians, combat controllers, instructors and other AFSOC personnel to better fine-tune the research methodologies to suit the end users (combat controllers and pararescuers) requirements. This technical report documents a series of laboratory and field investigations conducted under Congressional funding and the result of the various collaborations. The projects reported represent an enormous amount of data collection under a variety of environmental extremes and with a wide range of study participants.

Problem:

The goal of this effort was to improve training and sustainment programs for the Air Force Special Operations Command. Interventions are sought to enhance physical strength, endurance, fatigue resistance, and mental resilience. Over the course of this funding cycle, the effects of nutrition on human performance have been studied under various environmental conditions in the laboratory and austere environments. Similarly, the practical usefulness of biomarker detection during field environments has been evaluated. The overall purpose of this series of investigations has been to better understand strategies associated with training and nutritional interventions that optimize performance and enhance recovery from stressful conditions. In addition, the use of physiological monitoring devices for field data collection has been rigorously evaluated. Additional information has also been collected during events that better capture the human ceiling for energy expenditure and hydration demands.

The purpose of these series of investigations was to pool and consolidate research results from controlled laboratory study and field data collection efforts to provide information to the Air Force Special Operations Command in the areas of **Mission Preparedness and Recovery** and **Mission Performance**. These areas of interest serve as major headings below and are evaluated through specific research methodologies in and out of the laboratory.

Methods:

The specific methodology associated with the series of investigations has been separated into laboratory based and field based data collection efforts. These investigations focus on the areas of **Mission Preparedness and Recovery** and on **Mission Performance**. Details for each investigation are highlighted in the attached technical report and are specific to each project.

The methodology for this study series was carried out using mobile laboratory technologies and a newly developed research facility at the University of Montana. The research participants involved in these projects included recreationally active college aged males, highly trained endurance athletes, wildland firefighters and, military personnel associated with the Air Force Special Operations Command.

Our laboratory based study series included two nutritional intervention studies to evaluate the effectiveness of caffeine and a fenugreek extract. In addition, a caffeine study was completed after a short period of controlled, negative energy balance. We also completed a study to evaluate the performance and thermoregulation of individuals wearing training gear constructed from different technical fabrics. Our final laboratory based study was conducted to evaluate the effects of elevated ambient and core body temperature on muscle recovery.

The majority of our testing has been conducted during field operations under a variety of different climates, workloads, and durations. The goals of these field studies were to provide the highest possible external validity for better generalizability to the end user during extended military operations. These studies included field testing of various physiological monitoring systems, measures of total energy expenditure and water turnover during hostile, hot, cold, and high altitude environments. These projects also included time course evaluation of physiological adaptations and the biomarkers that associate with these positive changes within the skeletal muscle. Other biomarkers associated with overtraining were also evaluated for practical usefulness. In addition, specific nutritional interventions with military rations were evaluated to determine the effects on work performance and skeletal muscle recovery in wildland firefighters.

Solutions, Recommendations, Conclusions:

The findings demonstrate that mission performance can be altered by a variety of environmental factors that impede performance. However the environmental factors are of less concern compared to factors that can be altered and/or controlled by the warfighter. These include training status, approaches to training and also adequately meeting the nutritional and hydration demands of the required muscle work. The role of biomarker detection should receive additional attention as it relates to sample collection in the field. Biomarker sampling in a field setting requires special attention to collection procedure, sample handling and storage. This limits the practical usefulness of biomarker evaluation under "non-laboratory" conditions.

Nutritional provisions should be provided in hopes of allowing full recovery in between critical periods of training and/or mission objectives. The challenges to this relate to environmental concerns and the efficacy of traditional military rations to meet required muscle carbohydrate demand. Based on ultra-endurance research models, commonplace military operations and training scenarios do not approach the human ceiling for hydration and total energy requirements. In addition, some of the concerns related to physical preparedness of recruits can be addressed with more specific, individualized training that can elicit rapid physiological adaptations with intensified training. Moreover, "overtraining" during recruit training is not likely the result of physical demands but may be more influenced by other human factors.

Overall, the results from this 2-year effort indicate that to best understand and objectively evaluate the requirements on the human weapon system, a combination of tightly controlled laboratory investigations should be pooled with field research methodologies. This approach facilitates enhanced generalizability and data transfer to the end user.

Implications

The **political** implications associated with this 2-year research effort indicates that a diverse research agenda that maximizes both laboratory and field strategies ***can*** be implemented to serve the "real world" requirements of instructors, medical personnel and special combat operators. The current strategies for nutritional provision requires additional attention as our data clearly indicate that environment and established military ration systems may impair required muscle recovery. Similarly, training programs can be objectively evaluated with non-invasive physiological monitoring systems to maximize the physical benefits.

The other political implication indicates that other research models can serve as excellent surrogates to the rigors of military training and extended operations. This improves the diversity of data collection and increases the capability of partnering institutions that have limited access to military personnel.

Although research is costly, we have demonstrated that the combination of methodologies offers a unique approach and has **fiscal** implications for future data collection efforts. Through the combination of laboratory and field research, a productive, innovative research agenda can be established to address the physiological concerns of multiple end users. Our laboratory has become extremely efficient at collecting samples for a wide variety of operationally relevant settings. The most dramatic fiscal implications have to do with re-tooling some of the physical training strategies to better enhance the cellular weaponry of the modern warfighter. Although the cost input for these approaches is minimal, the benefits are where the cost effectiveness will be most observed. This approach will increase training pipeline production and warfighter safety and performance during extended operations. Other fiscal impacts may be associated with nutritional interventions and the need for structured supplemental feeding strategies that work under field conditions to better allow between mission muscle recovery.

The other political/fiscal implications for the state of Montana is that the Montana Center for Work Physiology and Exercise Metabolism is uniquely positioned to offer several agencies state of the art research facilities for cost effective data collection capabilities to address real time needs and concerns for the modern warfighter. Few laboratory facilities across the country have the capability to meet both of these demands using the cost effective strategies we have developed. These capabilities have been the direct result of several years combining field and laboratory methodologies and have been further established with the funding from this project series. This funding and support from AFRL and AFSOC have allowed us to establish a solid, state of the art facility that can serve as a dedicated university research center to address the physiological research needs of multiple military organizations.

Conclusions:

The following is a brief synopsis of some of our conclusions as they relate to each of our Lab-based and Field-based studies:

Lab Based Studies

Study 1: Glycogen Resynthesis and Exercise Performance with the Addition of Fenugreek Extract (4-hydroxyisoleucine) to Post-Exercise Carbohydrate Feeding.

Combined with our previous studies, these data suggest that the inclusion of a fenugreek extract will only enhance glycogen recovery after short term ($\geq$ 90 minutes) exercise. Therefore, the use of fenugreek is not recommended to enhance glycogen recovery after extended operations.

Study 2: Assessment of Muscle 8-Hydroxy-2'-Deoxyguanosine Levels in Relation to Repeated Sessions of Extended, Moderate Exercise in Aerobically Trained Individuals.

With recreationally trained males, 5 h of cycling exercise does not elicit pronounced DNA oxidation and cellular damage.

Study 3: A Recovery Drink Does Not Improve Glycogen Synthesis After Road Cycling in the Fed State.

When supplemental carbohydrate sources are consumed during endurance exercise that challenges muscle stores of glycogen, the timing and dose of post-exercise carbohydrate is less critical compared to exercise in the fasted state.

Study 4: Caffeine and Carbohydrate Supplementation During Exercise When in Negative Energy Balance: Effects on Performance, Metabolism, and Salivary Cortisol.

Caffeine when co-ingested with carbohydrate increases fat utilization and decreases non-muscle glycogen carbohydrate utilization over carbohydrate alone when participants are in negative energy balance.

Study 5: Effect of Post-Exercise Environmental Temperature on Glycogen Resynthesis.

If muscle glycogen is compromised and rapid recovery for subsequent exercise sessions is imperative, recovery in a cooler climate (at a lower core body temperature) will enhance skeletal muscle glycogen resynthesis compared to recovery in a hotter climate.

Study 6: Effects of Fabric Type on Core Temperature and Exercise Performance in the Heat.

While clothing choice is an important factor to consider when preparing to exercise or work in hot environments it seems that others factors can affect the body's ability to maintain homeostasis beyond the type of fabric covering the torso and upper arm.

Collectively, the data from these laboratory-based investigations indicate that to ensure adequate muscle recovery following glycogen depleting work/exercise sessions, there are factors surrounding environment and nutritional intake protocols. If rapid muscle recovery is warranted, a cooler environment will improve the rate of recovery. These data also suggest that the amount of and timing of ingestion of a post-exercise carbohydrate source is less critical if supplemental feedings are consistent during the period of work. When caffeine is ingested with carbohydrate, overall fat oxidation is improved. These data also indicate that individual fitness level is likely more predictive of heat tolerance compared to subtle differences in fabric design.

Field Based Studies

Study 1: First Strike Ration Elicits Similar Blood Chemistries as Meal, Ready to Eat During Three Days of Field Consumption.

The first strike ration is a lighter, easier to carry nutritional package that results in similar blood parameters compared to the established MRE.

Study 2: Proteinuria Induced by Arduous Work during Wildland Fire Suppression.

When dietary consumption and energy expenditure impair the maintenance of energy balance, total urinary protein excretion is increased. Moreover, the higher percent of body weight loss accompanied by dehydration after wildland firefighting may increase renal protein excretion. Although arduous work appears to acutely induce proteinuria as a result of a daily work output, whether long-lasting exposure to strenuous wildland firefighting activity induces a chronic glomerular and tubular damage still remains inconclusive.

Study 3: Muscle Glycogen Utilization During Wildfire Suppression

Wildland firefighting is an inimitable situation in which multiple factors, such as work output and dietary intake, not only vary from person-to-person, but also from day-to-day. This study demonstrates the variety of self-selected nutritional and activity habits of Wildlife Firefighters, and questions the adequacy of the MRE during sustained fire suppression operations in remote locations. Wildland firefighters need to feed aggressively throughout the work day and in the evenings with high CHO food sources in order to replenish depleted fuel sources.

Study 4: Effects of Beta Glucan on Symptoms of Upper Tract Infection in Wildland Firefighters.

The use of a beta glucan supplement may decrease the incidence of upper respiratory tract infection symptoms during multiple days of arduous work. This may provide for increased protection during extended periods of physical training and/or stress.

The data from studies 1-4 involving the wildland firefighter model more firmly establishes this subject population as an ideal parallel to military oriented training and/or operations. This data clearly indicates the benefits of supplemental feeding on exercise performance, mood state and safety. In addition, the design of ration packaging will influence the frequency of intake. When food components are accessible and require minimal preparation, the number of eating episodes increases as does the overall work output. Interestingly, when the MRE is too heavily relied on for multiple days of operations, muscle glycogen recovery is impaired, which will reduce exercise tolerance and the potential to perform at a high work rate. In regards to hydration and overall health, supplemental electrolyte added to water will reduce the required fluid intake during extended operations without compromising work output. In addition, the ingestion of a beta glucan supplement may reduce the risk and symptom profile for upper respiratory tract infections.

Study 5: Effects of Modafinil and Sleep Loss on Physiological Parameters.

Operators retained the physical stamina to complete physical tasks independent of Modafinil ingestion, particularly for tasks requiring muscular endurance. While incurring a higher oral temperature compared to placebo, hydration strain was similar between the Modafinil and placebo groups. Additionally, Modafinil did not elevate total energy expenditure compared to placebo.

Study 6: Accelerometry and Corresponding Physiological Stress During Air Force Special Tactics Officer Selection.

Special Tactics Officer Selection is physically and mentally demanding, with an estimated energy expenditure of approximately 4100 kcals per day. Although candidates are able to maintain fluid and energy balance, the stress associated with the course is evident based on salivary cortisol samples. It is anticipated that the course could be enhanced by the inclusion of additional physical tasks in the early evening hours. This may assist instructors in their decision making process of Officer selection.

Study 7: Comparison of Energy Expenditure and Activity Patterns During Seal Training Adventures Special Operations Forces Academy and U.S. Air Force Special Tactics Officer Selection.

The results indicate of whether the six day SOF Academy mimics the five day STO Selection, and readers should be hesitant to extend findings to long-term SOF training. In short, based on

activity monitoring and estimated energy expenditure, SOF Academy provides very similar physical stresses as STO Selection. SOF Academy would be an excellent model to conduct physiology research if parallels wanted to be made to STO Selection.

Overall, this series of studies 5-7 conducted using Air Force Operators and a surrogate SOF training camp indicates that the training exercises mimic other branches of SOF in terms of expected energy demands. If modafinil is prescribed for ground operations, it is not expected to dramatically increase the overall hydration or energy requirements of the operation. However, this requires additional research as the methodology for this investigation did not allow for periods of self-selected work. Other courses besides those offered for Officer selection may serve as an alternative research model when Air Force Operators are not available for research participation.

Study 8: Total Energy Expenditure, Energy Substrate Use and Hydration during an Ironman World Championships: A Case Study.

The results of this study indicate the extreme physiological strain associated with 10+ hours of physical work. The data further exceeds the recommendations for fluid intake requirements despite normal reduction in muscle glycogen. Environmental stress increases the hydration demand substantially during an extended work scenario that requires in excess of 9000 kcals to complete.

Study 9: Hydration Markers and Activity during Extreme Heat Stress and Ultra-Endurance Work: The Badwater Ultramarathon.

The current data demonstrate that individuals can maintain core temperature and proper hydration during endurance activities in high heat stress environments, if proper nutrition and fluids are available. Unfortunately, while homeostasis was maintained during activity in heat stress a reduction in work output was observed.

Study 10: Total Energy Expenditure, Body Water Turnover, and Hydration Status during the Western States 100: A Model for Extended Military Operations.

Based on this study total energy intake and hydration are the most limiting factor for participants in extended work scenarios. These athletes were able to maintain blood sodium concentrations compared to their pre-race concentrations, however, potassium and calcium concentration fell. This could be remedied by increased focus on more balanced electrolyte supplementation. The water turnover or the amount of water that is lost and replaced, of 17.9 ± 3.1 L demonstrates that the 17.9 L of water ingested was not sufficient to maintain hydration.

This series of studies 8-10 uses ultra-endurance research models to better describe the upper end of human physiology as it relates to hydration and total energy requirements. These data indicate that humans are capable of requiring up to 20 liters of fluid intake to maintain whole body hydration during 10-60 hours of muscle work that requires 9,000 – 19,000 kcals of energy expenditure.

Study 11: Applicability of the Actical to Measure Intensity and Duration of Activity During High Altitude Expeditions.

Activity monitors could become important tools for climb management purposes. The coordination of arterial saturation, sleep quality, AMS symptomology and stress accumulation (based on activity counts) could help climbers or medical support optimize climber rest: ascent schedules to decrease AMS symptoms and increase safety.

Study 12: Core Temperature During Consecutive Running Races.

While this study was primarily a pilot study for future investigation with core temperature monitoring during running, it was interesting that core temperature in the 5K and 1 Mile race did not reach the core temperature achieved in the 10K. Interestingly, the duration and intensity associate with the 10K event lead to the higher core temperatures. During training operations where core temperature may reach clinical significance and lead to medical emergencies, exercise intensity should be reduced. However, these data suggest that shorter periods of higher intensity work can be tolerated. It is only when the combination of intensity is paired with exercise durations >30 minutes, that core temperatures may increase to levels of moderate clinical significance.

Collectively, studies 11 and 12 offer evidence for the use of physiological monitors to more objectively evaluate periods of stress and/or extended work/training. The wide variety of physiological measurements that can be effectively measured under arduous and intense field environments may help expend the overall understanding as to the underlying physical demands.

Study 13: Adaptations to Periods of Intensified Training; Implications and Effectiveness of High Volume Training Camps.

The current data demonstrates that a short period of intensified training (less than 10 days) is enough to show favorable changes in metabolism. These changes will allow an individual to exercise/work longer and harder without decay of physical and mental capacities. The use of short intensified training strategies may provide useful in a periodized training program or as a pre military deployment protocol to quickly and effectively enhance human performance and thus upgrade the human weapon system.

Study 14: Effects of 21 Days of Intensified Training on Overtraining and Markers of Overtraining in Locally Competitive Cyclists.

During 21 days of intensified there was no decline in performance and only minimal changes in previously proposed markers of overtraining. By definition, the participants in this study did not reach a state of overreaching or overtraining since there was no decline in performance. The current data indicates that extreme periods of intensified training can be implemented in a safe manner without negative consequences in trained individuals. The key to success may be limiting other life stresses.

The most significant information from studies 13 and 14 indicate that previous measures of overtraining may be overestimated regarding their effectiveness. These data suggest that overtraining may not be a function of physiological strain but more a function of psychological issues surrounding increased requirements. Moreover, the metabolic adaptations associated with high-volume training appear to plateau early on (within 9 days in the present study). This has implications regarding the scheduling of pre-mission deployment strategies.

Lab Based Studies

Lab Based Study 1: Glycogen Resynthesis and Exercise Performance with the Addition of Fenugreek Extract (4-hydroxyisoleucine) to Post-Exercise Carbohydrate Feeding

D. Slivka, J. Cuddy, W. Hailes, S. Harger, and B. Ruby
Montana Center for Work Physiology and Exercise Metabolism
The University of Montana

Introduction:

Trigonella foenum graecum, commonly called fenugreek, is an annual plant originating in India and Northern Africa. The leaves and seeds are prepared into extracts or powders and used in traditional alternative medicine practice to treat several conditions including hyperglycemia and hyperlipidemia. The main component of this extract is the amino acid 4-hydroxyisoleucine (4-OH-Ile). Chronic 4-OH-Ile supplementation in the form of fenugreek has shown promising results in alleviating symptoms of diabetes in rodents (Raju *et al.*, 2001; Vats *et al.*, 2003; Mohamad *et al.*, 2004; Thakran *et al.*, 2004; Gad *et al.*, 2006; Mohammad *et al.*, 2006; Narender *et al.*, 2006) and in humans (Madar *et al.*, 1988; Sharma *et al.*, 1990; Sowmya & Rajyalakshmi, 1999; Abdel-Barry *et al.*, 2000; Gupta *et al.*, 2001). These studies have demonstrated a decrease in blood glucose (Madar *et al.*, 1988; Sharma *et al.*, 1990; Abdel-Barry *et al.*, 2000; Gupta *et al.*, 2001; Raju *et al.*, 2001; Vats *et al.*, 2003; Mohamad *et al.*, 2004; Gad *et al.*, 2006; Mohammad *et al.*, 2006), total cholesterol (Sharma *et al.*, 1990; Sowmya & Rajyalakshmi, 1999; Narender *et al.*, 2006), triglycerides (Sharma *et al.*, 1990; Gupta *et al.*, 2001; Narender *et al.*, 2006), free fatty acids (Narender *et al.*, 2006), and an increase in HDL cholesterol (Gupta *et al.*, 2001) and pancreatic β-cell insulin release (Sauvaire *et al.*, 1998). In addition, fenugreek has been shown to influence the insulin cell-signaling pathway (Broca *et al.*, 2004; Vijayakumar *et al.*, 2005), increasing glucose uptake in insulin sensitive tissues. These effects, along with the up-regulation of metabolic enzymes (Raju *et al.*, 2001; Vats *et al.*, 2003; Mohamad *et al.*, 2004; Gad *et al.*, 2006; Mohammad *et al.*, 2006) and increased muscle and liver glycogen storage (Vats *et al.*, 2003; Ruby *et al.*, 2005; Gad *et al.*, 2006), are consistent with the adaptations that occur with regular exercise training, suggesting that fenugreek supplementation may increase exercise capacity.

Muscle glycogen is a major energy substrate during exercise and thus is critical to performance (Hermansen *et al.*, 1967; Burke & Hawley, 1999). When the muscle glycogen concentration is reduced by a bout of exercise it must be resynthesized during recovery for individuals to perform at a high level during subsequent exercise sessions. Post-exercise intake of carbohydrate has been repeatedly shown to increase the rate of glycogen synthesis (Ivy, 2001). Essential to glycogen synthesis is glucose flux into the muscle, which can occur via insulin dependent and insulin independent (muscle contraction) pathways (Hayashi *et al.*, 1997; Goodyear & Kahn, 1998). The inclusion of protein or amino acids in post exercise carbohydrate feedings has been suggested to increase insulin (Zawadzki *et al.*, 1992) and thus promote glycogen synthase activity (Ivy *et al.*, 1988). However, other research has demonstrated no benefit with the addition of protein or amino acids to a high carbohydrate, post-exercise feedings (Carrithers *et al.*, 2000; van Hall *et al.*, 2000; Jentjens *et al.*, 2001). Since fenugreek is high in amino acids, especially 4-hydroxy-isoleucine, it is possible that this may exert an insulinotropic effect on glycogen storage. Our laboratory has recently shown that muscle glycogen resynthesis was

increased 67% during a four-hour recovery period after exhaustive exercise when fenugreek was added to a carbohydrate bolus. Additionally, there were no differences in the circulating insulin and glucose concentrations during the recovery period (Ruby *et al.*, 2005). These results indicate that fenugreek may act by a mechanism independent of insulin.

Research to date has focused on fenugreek supplementation in the hyperglycemic sedentary state and in a rodent model. To the authors' knowledge there has been only one published report in normoglycemic humans. This study demonstrated lower blood glucose and blood potassium just four hours after acute fenugreek supplementation (Abdel-Barry *et al.*, 2000). The impacts of fenugreek induced hypoglycemia and hypokalaemia on health and performance are unknown and merit further investigation. Evidence in mice suggests that fenugreek supplementation is beneficial to exercise performance in the normoglycemic animal. In these mice, swim time to exhaustion was increased with four weeks of fenugreek supplementation (Ikeuchi *et al.*, 2006). Thus, fenugreek may not only be effective for the treatment of diabetes, but may also serve as a potential ergogenic aid for the improvement of performance in athletes and healthy individuals.

Given the previous research, it is uncertain if increased muscle glycogen resynthesis during the early phase of recovery, with the addition of fenugreek during post-exercise carbohydrate feeding, would continue into the next day. Additionally, it is uncertain if increased muscle glycogen and/or other potential fenugreek actions would affect exercise performance on subsequent days. Thus, the purpose of this study was to investigate the effects of the addition of fenugreek to post-exercise carbohydrate feeding on muscle glycogen and subsequent exercise performance in normoglycemic trained male subjects.

Material and Methods:

Subjects

Eight trained male cyclists from the Missoula cycling community participated in this study (see table 1 for descriptive data). All subjects were given oral and written information about the experimental procedures and potential risks before giving written consent. All procedures conformed to the standards set forth by the *Declaration of Helsinki*, and the procedures were first approved by the University Institutional Review Board (Protocol #147-03, revised).

Preliminary Testing

Peak VO_2 was measured for each subject using a graded exercise protocol (starting at 95 watts and increasing by 35 watts every three minutes) on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). Expired gases were collected during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and analyzed at 15-second intervals. Body density was determined using hydrodensitometry and corrected for estimated residual lung volume. Net underwater weights were recorded using a digital scale (Exertech, Dresbach, MN). Body density was then converted to body composition using the Siri equation (Siri, 1993).

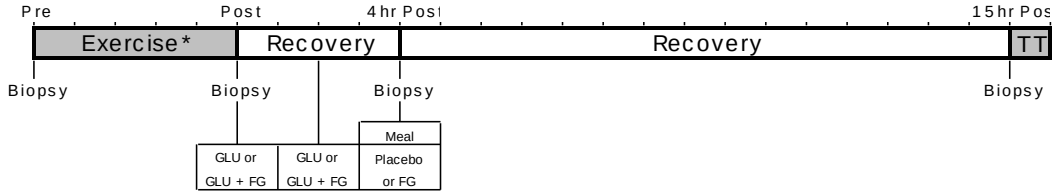

Figure 1. Schematic overview of the study design. * - supplemented with sports drink at beginning of each hour and expired gases collected at end of each hour, GLU - feedings of dextrose (1.8 g · kg^{-1} BW) plus placebo capsules, GLU + FG - feedings of dextrose (1.8 g · kg^{-1} BW) and the experimental supplement extracted from Fenugreek seeds. Meal - comprised a standardized meal, Placebo - placebo capsule, FG - fenugreek capsule, TT - simulated 40 km cycling time trial.

Table 1. Subject descriptive data (n = 8)

Age (yr)	28.6 ± 9.6
Height (cm)	180.0 ± 6.2
Weight (kg)	75.2 ± 8.2
Body Fat (%)	14.2 ± 3.4
Peak VO_2 (ml × kg^{-1} × min^{-1})	63.1 ± 5.9
VO_2 at LT (ml × kg^{-1} × min^{-1})	34.7 ± 4.6

Data are mean ± SD.

Design

Subjects completed a placebo controlled, double blind crossover design. The placebo trial (GLU) included feedings of dextrose (1.8 g · kg^{-1} BW per feeding) plus placebo capsules (micro crystallized cellulose), and the experimental trial (GLU + FG) included feedings of dextrose (1.8 g · kg^{-1} BW) and the experimental supplement (see Table 2) extracted from Fenugreek seeds (Technical Sourcing International, Missoula, MT). The feeding schedule was identical for GLU and GLU + FG trials and were isocaloric. Trial order was randomly assigned and counter-balanced with at least seven days between trials.

Experimental protocol

On the day of the trial, subjects arrived at the laboratory at 07.30 and consumed a standard breakfast containing 2854 kilojoules of energy (16.5 g fat, 117.1 g carbohydrate, and 17.1 g protein). The subjects relaxed in the lab until 10.00 to allow food to digest at which time a needle muscle biopsy from the *vastus lateralis* was performed (Pre). The subjects then cycled for 5 hours at an intensity equal to 50% of their peak power output (52.1±3.3% of VO_2 peak). During the exercise subjects were allowed to consume water *ad libitum* and were required to consume a volume of commercially available sports drink containing 0.12 g · kg^{-1}BW · hr^{-1} of carbohydrate at the beginning of each hour in an effort to better maintain a euglycemic state. During the final ten minutes of each hour, expired gases were collected in order to monitor exercise intensity relative to individual VO_2 peak.

Table 2. Amino acid profile and dose of the fenugreek supplement. Data were supplied by Technical Sourcing International, Missoula, MT and are relevant to the batch used in the current study.

	Relative Dose	Absolute Dose
Amino Acid	$mg \cdot kg^{-1}$ BW	mg
Arginine	0.07 ± 0.007	5.18
Aspartate	0.11 ± 0.011	8.14
Threonine	0.02 ± 0.002	1.48
Serine	0.07 ± 0.007	5.18
Glutamate	0.16 ± 0.016	11.84
Glycine	0.06 ± 0.006	4.44
Alanine	0.10 ± 0.010	7.40
Cyseine	0.06 ± 0.006	4.44
Valine	0.03 ± 0.003	2.22
Methionine	0.01 ± 0.001	0.74
Isoleucine	0.02 ± 0.002	1.48
Leucine	0.02 ± 0.002	1.48
Phenylalanine	0.05 ± 0.005	3.70
Ornithine	0.01 ± 0.001	0.74
Lysine	0.01 ± 0.001	0.74
Histidine	0.01 ± 0.001	0.74
Tyrosine	0.04 ± 0.004	2.96
4-OH-Ile	1.99 ± 0.202	148.00

Total amino acids (40%, 4-hydroxyisoleucine is approximately 25% of the 40%), alkaloids (35% primarily Trigonelline), protein/peptides (5%), fiber (1%), Ash (2%), moisture (12%), lipids, etc (5%). Data are mean ± SD.

Immediately following the five hour cycling exercise bout a second biopsy of the *vastus lateralis* was obtained from a separate incision made approximately 2 cm proximal to the initial biopsy site on the same leg (Post). Immediately after and again two hours following the Post biopsy, each subject received either GLU or GLU + FG. Four hours following the Post biopsy a third biopsy was taken (4hr Post) from a separate incision approximately 2 cm from the previous incision. The subjects then took another dose of placebo or experimental capsules along with a meal containing 3707 kilojoules of energy (22.3 g fat, 134.5 g carbohydrate, and 36.8 g protein). Subjects were allowed to consume provided snacks (3401±1661 kilojoules of energy). The snacks consumed were recorded so that these same snacks were consumed during the subsequent

trial. Therefore, each trial within an individual subject was isocaloric. Subjects were required to spend the night in the lab in order to closely control activity and diet.

On the following morning (15 hours after the completion of the 5 hour exercise bout), subjects awoke and a fourth muscle biopsy was obtained (15 hour Post) from a separate incision on the same leg 2 cm proximal from the previous biopsy. At this time subjects completed a simulated flat 40 km time trial on the same Velotron cycle ergometer utilizing Racer Mate custom course software (RacerMate Inc., Seattle, WA). See figure 1 for a schematic overview of the study design.

Biopsies

For each trial, biopsies were taken from the *vastus lateralis* muscle of the same leg in a randomized order using a 4mm Bergstrom percutaneous muscle biopsy needle (Bergstrom, 1962). Each successive biopsy on the same leg was obtained from a separate incision 2 cm proximal to the previous biopsy. After any excess blood and connective tissue or fat were removed, tissue samples were immersed in liquid nitrogen and stored at -80°C for later analysis.

Muscle glycogen analysis

Muscle glycogen was analyzed using an enzymatic spectophotometric method. Samples were weighed (12.8 ± 4.6 mg wet weight) upon removal from a -80°C freezer and placed in 0.5 ml, 2N HCl solution. The sample solutions were weighed, incubated for two hours at 100°C in an oven, re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5ml of 0.67 NaOH was added. A volume of this muscle extract (20μl) was added to 1 ml of Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd) and read on a spectrophotometer at 340nm. Muscle glycogen was then calculated using the extinction co-efficient of NADH. Muscle glycogen concentrations are expressed in mmol · kg^{-1} wet weight of muscle.

Statistics

Muscle glycogen was analyzed using a repeated measure ANOVA (trial x time). Time trial performance was analyzed using a two-tailed paired t-test. A probability of type I error less than 5% was considered significant ($p<0.05$). All data are reported as means ± SD.

Results:

Muscle glycogen

There was no trial x time interaction for the measure of muscle glycogen ($p>0.05$), indicating that the rate of muscle glycogenolysis during the 5 hour exercise and the rate of muscle glycogen resynthesis after exercise were similar between the GLU and GLU + FG trials. A main effect for time was observed ($p<0.05$), demonstrating that muscle glycogen decreased with the 5 hour exercise bout and then was increased at 4 hours post exercise and on the next morning (15 hour post) after exercise. See figure 2.

Time Trial Performance

There was no difference ($p>0.05$) in average power output (221±28 vs. 213±16 watts) or time to completion (69.7±3.7 vs. 70.5±2.2 minutes) of the simulated flat 40 km time trial between GLU

and GLU + FG, respectively (Figure 3).

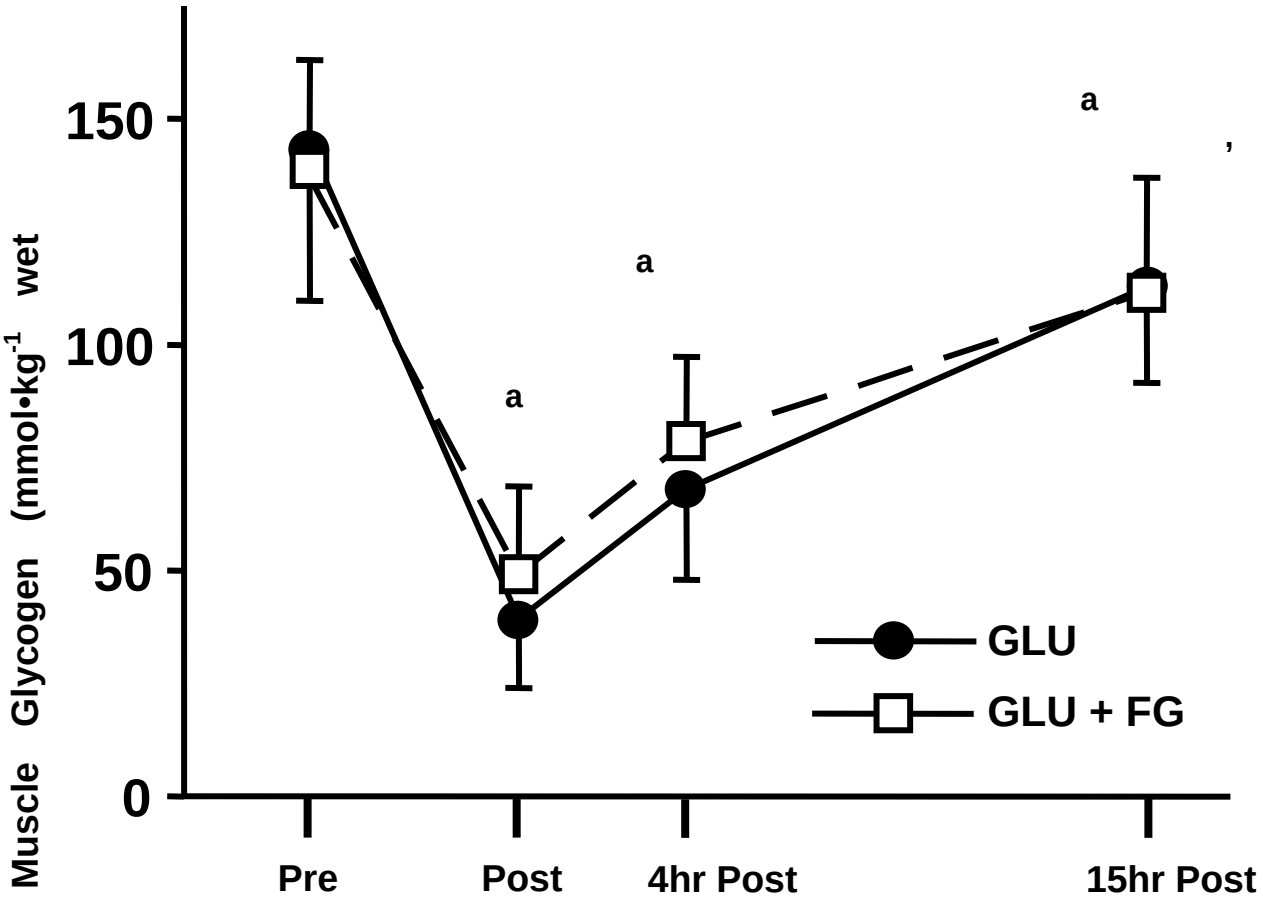

Figure 2. Changes in muscle glycogen content with five hours of cycling exercise (Pre – Post) and recovery (4 hour Post, 15 hour Post) in response to post exercise glucose (GLU) and glucose with fenugreek (GLU + FG) post exercise feedings. a – $p < 0.05$ from pre (main effect for time). b – $p < 0.05$ from post (main effect for time). c – $p < 0.05$ from 4 hour Post (main effect for time). Data are mean ± SD.

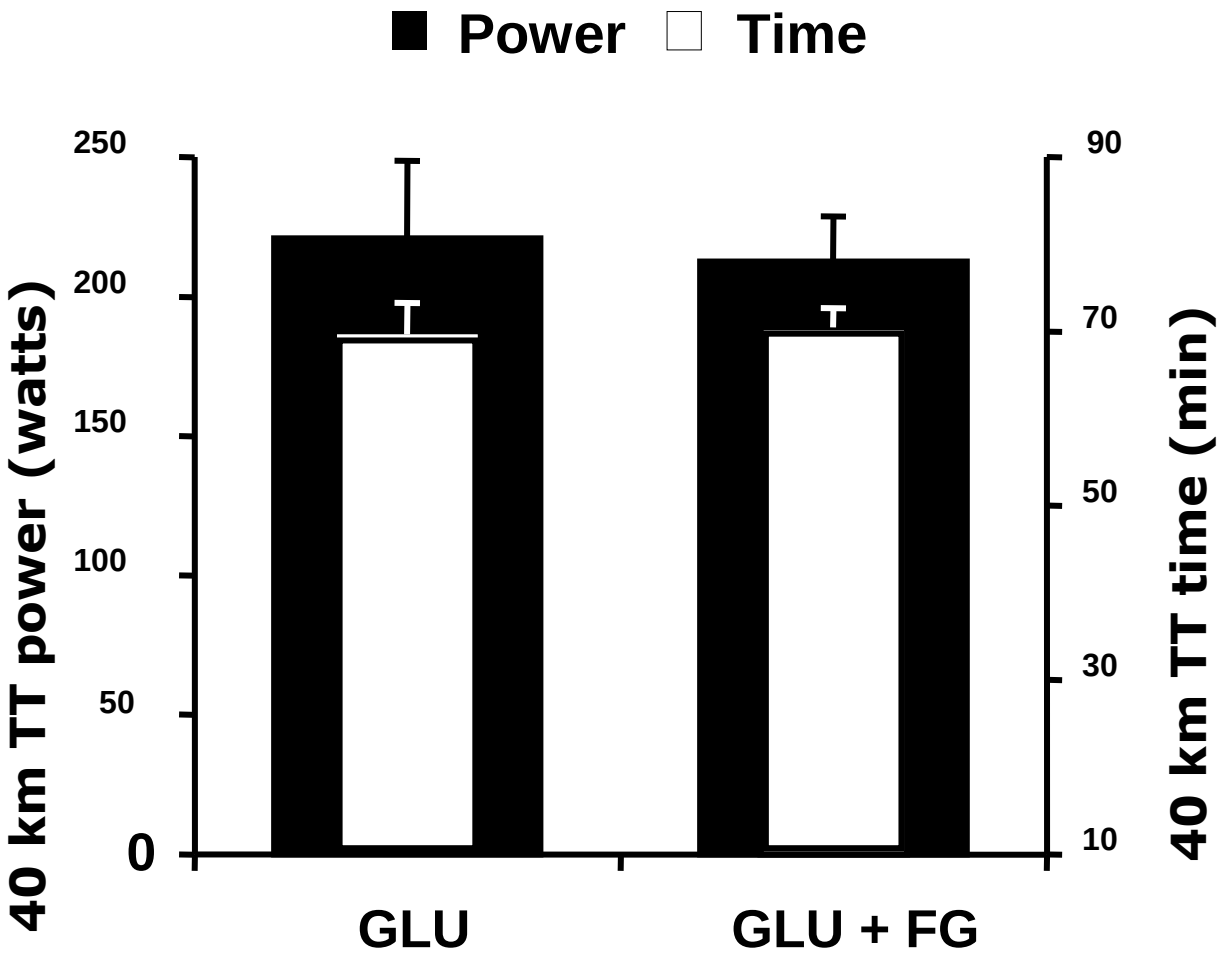

Figure 3. Simulated 40 km time trial performance (left axis power, right axis time) with glucose (GLU) and glucose with fenugreek (GLU + FG) post exercise feedings. Data are mean ± SD.

Discussion:

The current study was designed to simulate a common challenge that endurance athletes encounter. Time for optimal recovery is often not available during peak training periods and multi-day races. Our previous research indicated that the combination of fenugreek with carbohydrate promoted a 63% higher rate of post-exercise muscle glycogen resynthesis compared to carbohydrate alone (Ruby *et al.*, 2005). The resynthesis of muscle glycogen is an important component of recovery for endurance athletes, thus our previous results would suggest that subsequent performance would also be increased. However, no marker of exercise performance was included in this earlier study. The purpose of the current study was to investigate the addition of fenugreek to post-exercise carbohydrate feedings on glycogen resynthesis and subsequent exercise performance in normoglycemic trained males. In light of

the previous research on the beneficial effects of fenugreek, including our own, the novel findings of the current study were that fenugreek supplementation had no effect on glycogen resysnthesis or on subsequent exercise performance in response to this exercise protocol.

It is not obvious to the authors why the current results differ from that of our previous study (Ruby *et al.*, 2005). While the initial four hours after exercise were identical between the studies, the exercise bout itself was quite different. The previous study included 90 minutes of high intensity cycling intervals, while the current study involved five hours of low intensity steady state cycling. Additionally, the current study allowed subjects to consume small amounts of carbohydrate during exercise. It is interesting to note that, despite these differences in exercise protocol, the post exercise and the 4 hour post-exercise muscle glycogen concentrations are similar in response to the placebo trial for each of the two studies.

The actions of fenugreek, when combined with high intensity exercise, may produce a synergistic effect to increase the rate of muscle glycogen resynthesis. Due to the broad range of reported effects of fenugreek, the mechanism of action is likely multi-faceted. A series of experiments by Vijayakumar *et al*. (Vijayakumar *et al.*, 2005) have characterized a mechanism of action for fenugreek aided glucose uptake. These experiments demonstrated that fenugreek led to increased tyrosine phosphorylation of the p85 subunit of phosphatidylinositol 3-kinase (PI3-K). The downstream target of PI3-K, protein kinase C (PKC), was further activated. However, no effect on the other downstream target of PI3-K, protein kinase B (Akt), was noted. In this model, fenugreek leads to increased glucose transport independent of Akt via increased fusion of GLUT4 vesicles that are within 250 nm of the plasma membrane (Gonzalez & McGraw, 2006). While little is known about the mechanism associated with contraction mediated GLUT4 translocation, insulin stimulated GLUT4 translocation is regulated by Akt (Gonzalez & McGraw, 2006). Akt phosphorylation and/or activity has been shown to increase as a result of exercise (Thorell *et al.*, 1999; Turinsky & Damrau-Abney, 1999; Nader & Esser, 2001; Sakamoto *et al.*, 2004). Moreover, the magnitude of the exercise induced increase in Akt may be a factor of exercise intensity (Sakamoto *et al.*, 2004). Thus, increased Akt activity during high intensity exercise may increase GLUT4 translocation and thus increase glucose transport. Additionally, Akt has been implicated as a possible candidate for mediating glycogen synthesis (Peak *et al.*, 1998). These factors lead to the possibility that increased Akt activity as a result of high intensity exercise, but not low intensity exercise, may be necessary to induce fenugreek promoted glucose transport and subsequent rate of muscle glycogen resynthesis. More studies are needed to clarify exercise induced glucose uptake signaling and the possible effects of fenugreek supplementation.

The current research used normoglycemic healthy male athletes given acute post-exercise doses of fenugreek. The majority of research involving fenugreek includes: 1) rodentstudies, 2) chronic dosing, 3) the use of hyperglycemic (diabetic) models, and 4) no exercise intervention. Other than the previous report from our laboratory (Ruby *et al.*, 2005), the only investigation in an acute normoglycemic human model reports a 13% decrease in blood glucose and a 14% decrease in potassium levels four hours after fenugreek ingestion (Abdel-Barry *et al.*, 2000). These authors noted that approximately one third of the subjects experienced symptoms such as feelings of hunger, increased micturition frequency, and dizziness. Furthermore, the decrease in blood potassium levels at rest may have ominous effects on the heart, nerves, and skeletal

muscle. During exercise, the redistribution of potassium out of the blood and back into the muscle cells would be beneficial. Under normal exercise conditions, there is a net potassium release from contracting muscles. The resulting increased extracellular potassium and decreased intracellular potassium contributes to muscle fatigue by depolarizing the resting membrane potential (Clausen & Everts, 1991; Lindinger & Heigenhauser, 1991). If fenugreek exerts an effect on the Na/K ATPase to redistribute potassium to intracellular compartments muscular fatigue may be reduced. Swim time to exhaustion in mice has been shown to increase with fenugreek supplementation (Ikeuchi *et al.*, 2006). While potassium levels were not measured, it was concluded that swim time to exhaustion was improved due, at least partially, to increased fat utilization.

In the current study, performance was not improved with fenugreek supplementation. The performance trial was conducted eleven hours after the last dose of fenugreek was administered. This lapse of time may be too long in order for the acute reduction in potassium to have an effect, while the acute doses of fenugreek supplementation may not have been continued long enough for the chronic effect of increased fat utilization to affect exercise performance. We had hypothesized that increased glycogen availability in the GLU + FG group would lead to improved exercise performance. However, since muscle glycogen stores were similar between GLU and GLU + FG, exercise performance did not improve.

Conclusions:

The current research demonstrated no effect with the addition of fenugreek to post-exercise glucose feeding on muscle glycogen resynthesis or exercise performance. The contrast of this with previous work (Ruby *et al.*, 2005) may be partially explained by the differences in exercise intensity and duration and their effect on cellular signaling cascades, ultimately leading to glucose transport and subsequent glycogen synthesis. Alternatively, the outcome of this or our previous study may have been fortuitous. However, the impressive array of reported effects of fenugreek are difficult to ignore and it is possible that effects should be interpreted with specificity and not generalized. More research is needed in order to understand the interactions that fenugreek may have in the normoglycemic exercising human and the conditions required to induce physiological effects.

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Lab Based Study 2. Assessment of Muscle 8-Hydroxy-2'-Deoxyguanosine Levels in Relation to Repeated Sessions of Extended, Moderate Exercise in Aerobically Trained Individuals

Nobuo Yasuda[1], Celeste Bolin[2], Fernando Cardozo-Pelaez[2], and Brent C. Ruby[1]
Montana Center for Work Physiology and Exercise Metabolism[1]
Biomedical and Pharmaceutical Sciences[2]
The University of Montana
Missoula, MT
59812

Introduction:

It has been demonstrated that increased levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) serve as a biomarker of oxidatively damaged DNA by ROS and RNS in the mitochondria [Kasai & Nishimura 1984; Loft et al. 1992]. The magnitude of the oxidative DNA damage appears to rely on the balance of free radical generation and the capability of antioxidant systems [Sato et al. 2003]. Repeated exercise and training are considered to attenuate the oxidative stress by enhancing antioxidant defense mechanisms including antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase [Sato et al. 2003; Sen 1995; Wang & Huang 2005]. In line with those phenomena, DNA repairing enzymes such human 8-oxoguanine DNA glycosylase1 (hOGG1) and oxidized purine-nucleoside triphosphatase (hMTH1) can be augmented after endurance exercise in order to protect against exercise-induced DNA damage [Radak et al. 2003; Sato et al. 2003].

With regard to muscle 8-OHdG level in humans, only one study has described augmented 8-OHdG levels in quadriceps femoris muscle following a single bout of eccentric exercise [Radak et al. 1999b]. In contrast, some rat studies have reported attenuated 8-OHdG levels in gastrocnemius muscle after 8 weeks of treadmill running [Radak et al. 2002] and 9 weeks of swimming [Radak et al. 1999a]. A canine study has shown no changes of 8-OHdG levels in gastrocnemius muscle after 7 h of treadmill running compared with the control group [Okamura et al. 1997]. Taken collectively, although the changes of blood or urinary 8-OHdG are well documented, to date, there have been no reports examining the alterations in muscle 8-OHdG level following endurance exercise in humans.

The purpose of this study was to determine the effects of repeated, extended exercise on muscle DNA damage in endurance athletes. It was hypothesized that there would be no differences at the muscle levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) between two exercise sessions.

Methods

Subjects

Seven trained males from the Missoula cycling community served as the subjects (Age: 29.9±9.9 yr, Height: 181.0±6.5 cm, Weight: 76.1±7.2 kg, %V O_2peak at lactate threshold:

57.1±7.2%). When subjects' muscle samples at some of time points were not sufficient to determine muscle 8-OHdG, the time points were excluded. None of the subjects reported a history of regular exogenous antioxidant supplementation or other drugs with antioxidant properties. All participants provided written informed consent prior to the current study. The protocol of the present study was reviewed and approved by the University Institutional Review Board (Protocol #147-03, revised).

Preliminary Exercise Testing

VO_2peak and lactate threshold were selected to evaluate the subjects' fitness levels. Hydrostatic weighing was used to determine percent body fat.

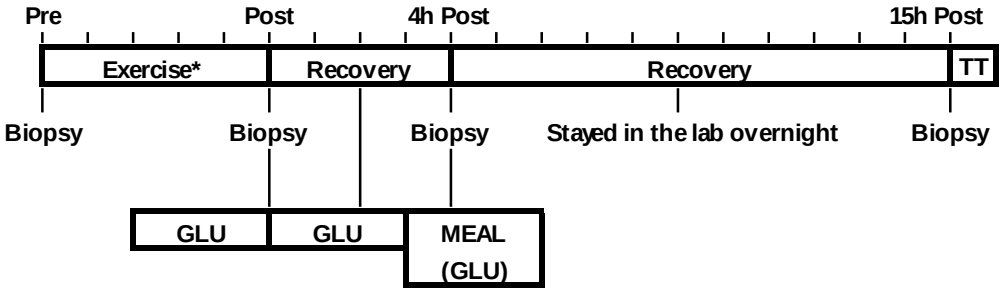

Figure 1. The schematic diagram of the experimental procedures. *Time point supplemented with sports drink at beginning of each hour and expired gases collected at end of each hour. GLU: feedings of dextrose (1.8 $g \cdot kg^{-1}$ body weight), PRE: before exercise, MEAL: solid food meal, and TT: simulated 40 km cycling time trial.

Experimental Procedure

Figure 1 presents a schematic diagram of the experimental procedure in the present study. All participants reported to the laboratory at 0730 on the day of the trial. Each participant was instructed to consume a standard breakfast consisting of 2854 kilojoules of energy (16.5 g fat, 117.1 g carbohydrate, and 17.1 g protein). After breakfast, the subjects settled down in the lab until 1000 to allow food to digest at which time a muscle biopsy from the *vastus lateralis* was taken (Pre) [Slivka et al. 2007]. The subjects then performed 5-h cycling exercise at an intensity of 50% of their peak power output (52% V O_2peak). In our pilot study, moderately trained individuals could not maintain more than 55-65% of cycling exercise for a long-term duration. Thus, 5 h of cycling exercise at 52%VO_2peak (closer to Tlact) was chosen in the present study, which was considered as an upper limit for extended, steady-state exercise. During exercise, subjects were instructed to take water *ad libitum* and to consume a standard sports drink consisting of 0.12 $g \cdot kg^{-1}$ body $weight \cdot hr^{-1}$ of carbohydrate. Carbohydrate sources were consumed at the beginning of each hour. In order to monitor exercise intensity relative to individual VO_2peak, expired gases were analyzed during the final ten minutes of each hour.
Immediately following the five hour cycling exercise bout, a second biopsy was obtained from a separate incision made approximately 2 cm proximal to the initial biopsy site on the same leg

(Post). Four hours following the Post biopsy, a third biopsy was carried out (4-h Post) at a separate site approximately 2 cm from the previous incision. All subjects then consumed a meal composed of 3707.0 kilojoules of energy (22.3 g fat, 134.5 g carbohydrate, and 36.8 g protein). Subjects were allowed to ingest provided snacks (3401±1661 kilojoules of energy), which were also consumed during the subsequent trial. Subjects stayed in the lab overnight in order to closely control activity and diet.

On the following morning (15 hours after the completion of the 5 hour exercise bout), a fourth muscle biopsy was obtained (15-h Post) at a different incision made 2 cm proximal from the previous biopsy. Subjects then completed a simulated flat 40km time trial on the same Velotron cycle ergometer with RacerMate® custom course software (RacerMate Inc., Seattle, WA).

Determination of Muscle 8-OHdG Levels

Muscle biopsies were obtained from the *vastus lateralis* muscle of the same leg in a randomized order for each trial [Slivka et al. 2007]. Muscle samples were immediately immersed into liquid nitrogen after getting rid of any excess blood, connective tissue or fat and stored at –80°C until subsequent analyses.

The determination of 8-OHdG levels in the muscle tissue was based on the protocol previously described by Bolin et al. [Bolin et al. 2004]. 8-OHdG and 2-dG were resolved by HPLC with a reverse phase YMCbasic column (4.6 mm × 150 mm; particle size 3 μm)(YMC Inc., Wilmington, NC) and quantified using a CoulArray electrochemical detection system (ESA Inc., Chelmsford, MA).

Statistical Procedure

A two-way Analysis of Variance (ANOVA) with repeated measures (a mixed design) was performed on muscle 8-OHdG levels. The acceptable level of statistical significance was set at $p \leq 0.05$. All results are described as mean ± SD.

Results:

No significant differences in muscle 8-OHdG levels were observed between sessions over the experimental time course (Figure 2).

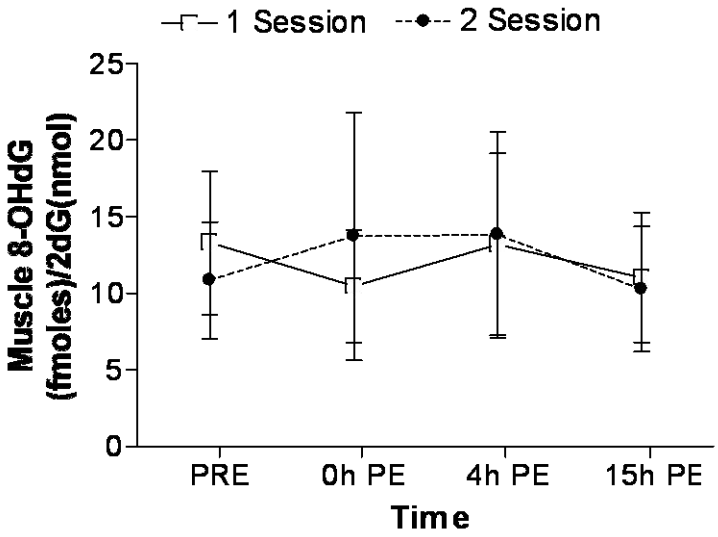

Figure 2. Muscle 8-hydroxy-2'-deoxyguanosine (8-OHdG) expressed relative to deoxygunosine (2dG)[8-OHdG (fmoles)/2dG(nmol)] between first and second sessions. PRE: before 5-h exercise, PE: post 5-h exercise. All data express mean±SD (n=4-5).

Discussion:

The main results in the present study demonstrated that extended, moderate exercise closer to lactate threshold did not induce exercise-induced DNA damage at the muscle level. To our knowledge, this study is the first to measure oxidative DNA damage in muscle, evaluating levels of 8-OHdG after repeated sessions of prolonged exercise in humans. It appears that a long-term, moderate exercise is beneficial in order to enhance the capacity of antioxidant system.

Muscle 8-OHdG Levels

It has been suggested that exercise induces ROS and RNS generation in the mitochondria, which leads to oxidative DNA damage in the skeletal muscle [Radak et al. 1997]. Previous studies have demonstrated the more prominent antioxidant capacity of Type I fibers (slow twitch, oxidative) compared with that of Type II fibers (fast twitch, glycolytic) [Loft et al. 1992; Radak et al. 1997; Radak et al. 2001]. Some human studies have reported that endurance exercise and training, accompanied with a more prominent involvement of Type I fibers, promotes the prevention of nuclear DNA damage by up-regulating the repairing enzyme activity such as hOGG1 [Radak et al. 2003] and hMTH1 [Sato et al. 2003]. This is supported by the fact that in endurance-trained individuals, the Type I fibers are more noticeable because of abundant mitochondrial density compared with the Type II fibers [Coyle 1995]. Thus, trained athletes or individuals who are regularly involved in endurance training may have an enhanced adaptive response, which results in a protective effect against oxidative stress [Radak et al. 2003] and attenuates the extent of apoptosis (e.g., DNA fragmentation and caspases activity) [Peters et al. 2006]. However, whether or not DNA damage directly results from oxidative damage, or whether it is derived from a part of the process of apoptosis remains elusive [Mars et al. 1998].

In terms of muscle 8-OHdG levels in humans, only one study has reported increased DNA

damage in quadriceps femoris muscle after a single bout of eccentric exercise [Radak et al. 1999b]. However, a canine study has described no alterations of the 8-OHdG levels after 7 h of treadmill running [Okamura et al. 1997]. Some rat studies have shown the reduction of the 8-OHdG levels in gastrocnemius muscle after 8 weeks of treadmill running [Radak et al. 2002] and 9 weeks of swimming [Radak et al. 1999a] compared with the control group. In the current study, no significant changes in muscle 8-OHdG levels were noted at any time point.

Previous findings described oxidatively damaged DNA in the lymphocytes of relatively untrained subjects 24-h post-exercise [Hartman et al. 1994; Mars et al. 1998; Niess et al. 1998; Tsai et al. 2001]. In contrast, Peters et al. [2006] found no significant increases in DNA migration as a measure of damage at 3 h post-exercise following 2.5-h exercise trials. Moreover, Mars et al. [1998] described significant increases in both DNA strand breaks and apoptotic cells in untrained subjects, immediately after incremental exercise protocol to exhaustion, but not 24 and 48 hours after exercise. Taken collectively, the discrepancies between previous studies of DNA damage may be due to the variability of exercise intensity and duration, fitness level of the research participants [Peters et al. 2006], differences of sampled tissues (e.g., blood or muscle), sampling time and assay (e.g., HPLC or ELISA).

Conclusions:

There was a tendency that repeated sessions of extended, moderate exercise closer to Tlact did not induce oxidatively damaged DNA as measured by 8-OHdG at the muscle level in aerobically trained individuals. These findings indicate that a long-term, moderate exercise is beneficial to balance a damage against a recovery with respect to muscle protein turnover.

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Lab Based Study 3: A recovery drink does not improve glycogen synthesis after road cycling in the fed state.

D. Reinert, D. Slivka, J. Cuddy, and B. Ruby
Montana Center for Work Physiology and Exercise Metabolism
The University of Montana

Supplemental Funding: Gatorade Sports Science Institute

Introduction:

The majority of research examining muscle glycogen recovery in response to cycling has been conducted under laboratory conditions with subjects in a fasted state, without CHO available during exercise. In the present study we aimed to create a more realistic scenario; therefore, subjects were fed CHO before, during, and after each ride, which reflects a common nutritional practice for cyclists. Moreover, the development of portable power meter devices allow for accurate measures of real time power output during road cycling[3]. The cycling trials in the present study were performed outside on a set course with road bikes equipped with a rear hub power meter (Saris Cycling, Madison, WI).

The primary purpose of the present investigation was to determine the efficacy of a supplemental carbohydrate-protein (CHO-PRO) recovery beverage when commonplace nutritional supplementation was supplied during a 62 km road ride. A secondary purpose was to demonstrate the effectiveness of bicycle integrated power meter to standardize the physiological responses to repeated bouts of road cycling.

Methods:

Subjects
Eight recreationally active male cyclists from the Missoula cycling community (mean ± SD: age 25 ± 4 yrs, mass 69.3 ± 5.2 kg, VO_2 peak 4.5 ± 0.4 L/min, body composition 10.8 ± 2.8% body fat) participated in this study. Prior to data collection, the research procedures were approved by the University Institutional Review Board (Protocol #173-05, revised). Subjects were informed of all experimental procedures and risks associated with the study and provided written consent prior to participation.

Preliminary testing
Peak oxygen uptake (VO_2 peak) was determined for each subject using a ramp protocol (25 W $\cdot min^{-1}$) on an electronically braked cycle ergometer (Velotron, Seattle, WA). Expired gases were collected and analyzed at 15-second intervals during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT). Body composition was determined using hydrodensitometry. Body weight was recorded on a dry weight scale (Befour Inc., Cedarburg, WI). Body density was measured using a calibrated underwater digital scale (Exertech, Dresbach, MN) and converted to percent body fat using the Siri equation[21].

Experimental Trials

All subjects participated in a placebo and experimental trial in a double-blind, randomized counter-balanced design with at least 6 days between trials. After an overnight fast, subjects consumed a breakfast at 0715 (107g CHO, 16g protein, and 11.6g of fat). At 0930 a baseline blood sample was obtained. Immediately after the blood collection, a muscle biopsy was obtained from the midsection of the *vastus lateralis* muscle with a 5mm Bergstrom needle. Muscle samples were dissected of any visible connective tissue and immediately frozen in liquid nitrogen. Samples were stored at – 80°C until assayed for total muscle glycogen. At 1000 subjects consumed a food bar consisting of 38g CHO, 15g protein, and 5g fat. At 1015, subjects began the 62 km ride with 600 ml of water and 600 ml of a commercial carbohydrate beverage (6% CHO solution) in separate bottles mounted to their road bike. Subjects completed a pre-determined course at a self-selected intensity similar to a typical training ride. Time, velocity, distance, heart rate (HR) and power (watts) were displayed and recorded during the ride with a mounted cyclometer (PowerTap Pro, Saris Cycling, Madison, WI). For the second trial, subjects were provided with a detailed ride script based on the first trial to ensure similar intensities for the second road cycling trial (Figure 1). During both trials, at minutes 45-55 and minutes 90-100, subjects were instructed to increase their power output for 10-minutes to simulate a higher-intensity interval common in cycling training. Immediately after each ride, a post exercise blood sample was collected and a second biopsy was obtained approximately 1 cm proximal to the first biopsy on the same leg. At 30 minutes post-exercise, subjects ingested 360 ml of a recovery beverage containing 40g CHO, 20g protein (CHO-PRO) or a similarly flavored placebo (PL). Additional blood samples were collected at 1, 2.5, and 4 hrs post-exercise. Two hours post-exercise on both trials, subjects consumed a standardized meal (165g CHO, 40g protein, and 7g fat). A final muscle biopsy was obtained at 4 hours post-exercise from the vastus lateralis approximately 1 cm proximal from the second biopsy on the same leg.

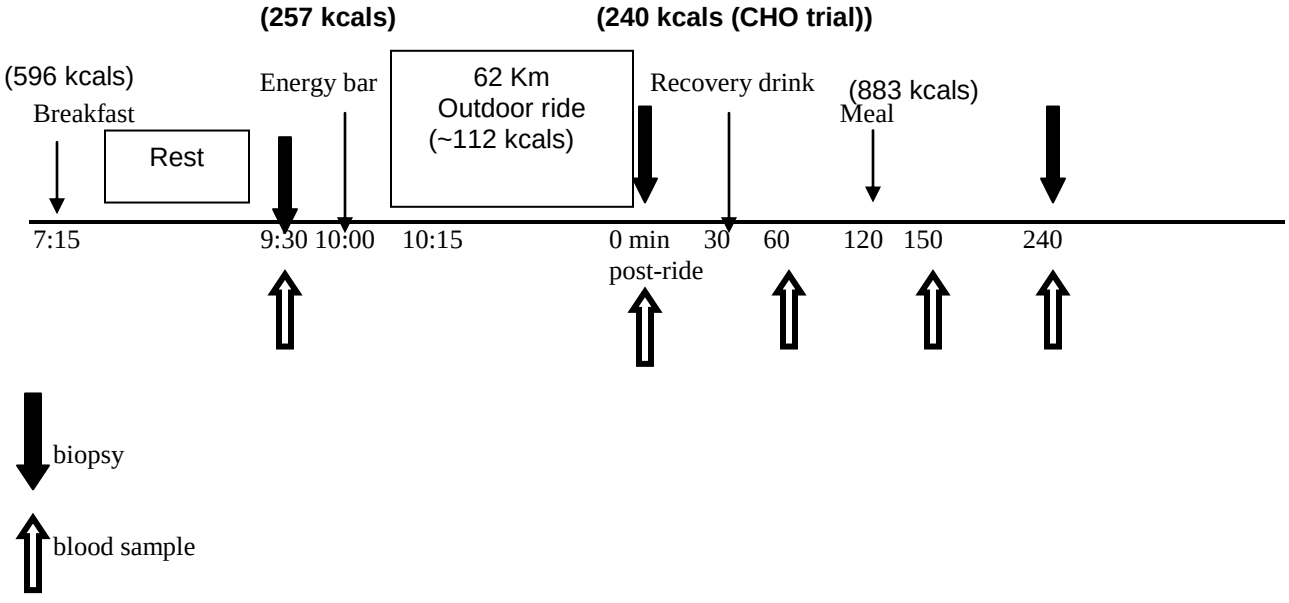

Figure 1. Experimental events and schedule.

Blood analysis
Blood samples were analyzed in duplicate for glucose using an enzymatic spectrophometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Insulin was analyzed in duplicate using an ELISA method (EIA2935, DRG International).

Muscle glycogen analysis
Muscle glycogen was analyzed using an enzymatic spectrophometric method after tissue preparation. Samples (25 ± 3.2mg wet weight) were weighed, placed in 1 ml, 1 N HCL solution, and homogenized using a manual mortar and pestle tissue grinder. Samples were incubated at 95.6°C for three hours. After the incubation, 0.5 ml, 1 N NaOH was added to 0.5 ml of tissue sample to normalize pH. Samples were analyzed in triplicate against known glycogen and glucose controls run simultaneously. Muscle glycogen concentrations were expressed in mmol $\cdot kg^{-1}$ wet wt. muscle.

Statistics
Ride data (ride duration, average power output, HR, CHO intake, and intensity) were analyzed using 2-tailed dependent t-tests. Blood glucose, insulin, and muscle glycogen concentrations were analyzed using a 2-way ANOVA with repeated measures. A bonferroni adjustment was made for multiple comparisons to identify differences when a significant F-ratio was identified in an ANOVA. Statistical significance was established using an alpha of $p < 0.05$. Data are reported as mean ± SE.

Results:

Ride Data
There were no significant differences between the PL and CHO-PRO trials for ride duration, power, or HR (Table 1, Figure 2).

Table 1. Descriptive data for both trials (mean ± SE).

	PL	**CHO-PRO**
Duration (min)	122.4 ± 2.1	123.8 ± 1.8
Power (avg. watts)	210 ± 10	212 ± 9
HR (bpm)	148 ± 3	148 ± 3

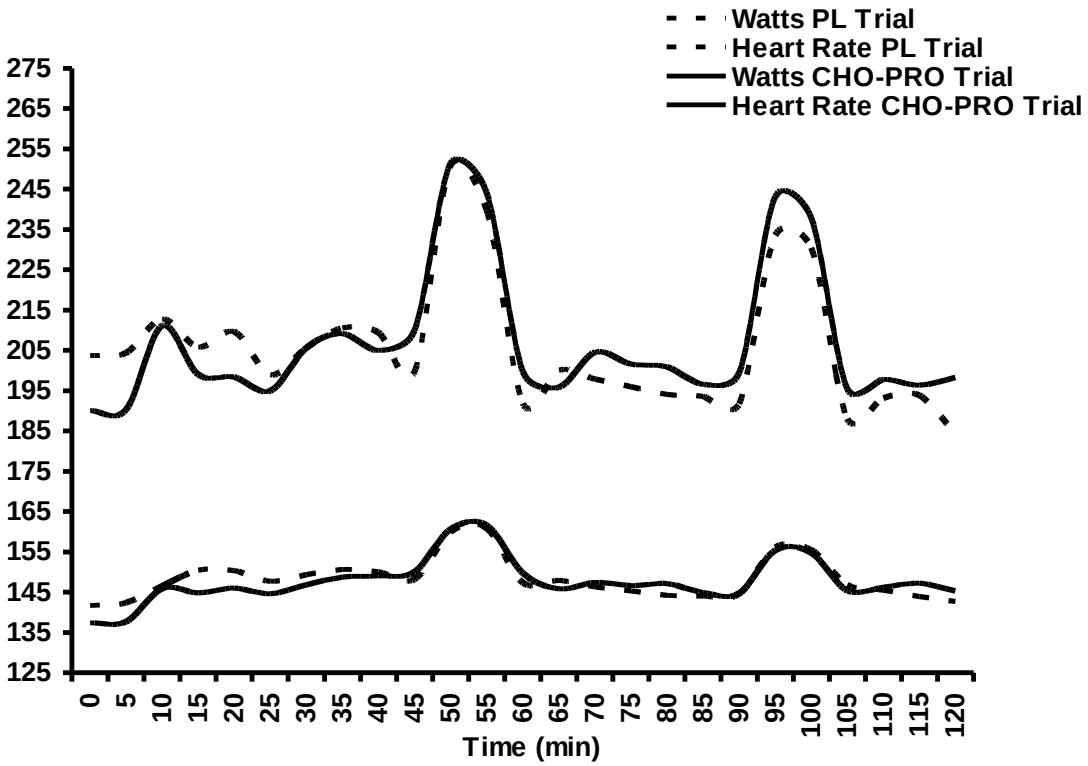

Figure 2. Mean heart rate and power for both trials (5 min averages).

Serum glucose

There were no significant differences between trials in pre, post, 2.5 hours post, or 4 hours post-exercise blood glucose. However, at 1 hour post-exercise, the CHO-PRO trial demonstrated a higher blood glucose compared to PLA ($p<0.05$). The CHO-PRO trial showed a significant increase over post-exercise blood glucose at 1 and 2.5 hours post exercise, returning to post exercise values by 4 hours post. In contrast, the PLA trial demonstrated a blood glucose concentration similar to post exercise at 1-hour post ride, an elevated blood glucose at 2.5 hours post-exercise, and a return to post exercise levels by 4 hours post (Figure 3).

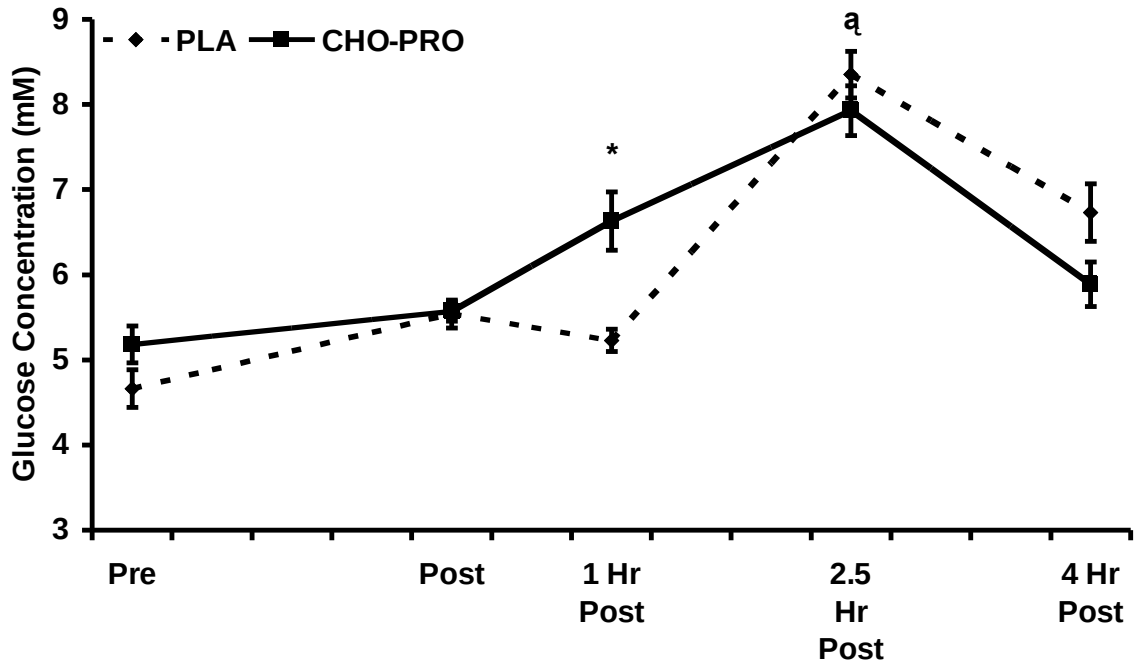

Figure 3. Serum glucose concentrations (mM) during the exercise and recovery periods. *, $p<0.05$, CHO-PRO vs. PL. †, $p<0.05$ vs. post (PLA); ‡, $p<0.05$ vs. post (CHO-PRO).

Serum insulin

There were no significant differences between trials in pre, post, or 2.5 hours post-exercise insulin. However, at 1 hour post-exercise, the CHO-PRO trial demonstrated significantly higher insulin compared to PLA trial at 1 hour post exercise while the PLA trial demonstrated a significantly higher insulin at 5 hours post-exercise. The CHO-PRO trial showed significantly elevated insulin at hours 1, 2.5 and 4 post-exercise compared to post exercise values. In contrast, the PLA trial showed an unchanged insulin concentration at 1-hour post and elevated values at 2.5 and 4 hours post when compared to post-exercise (Figure 4).

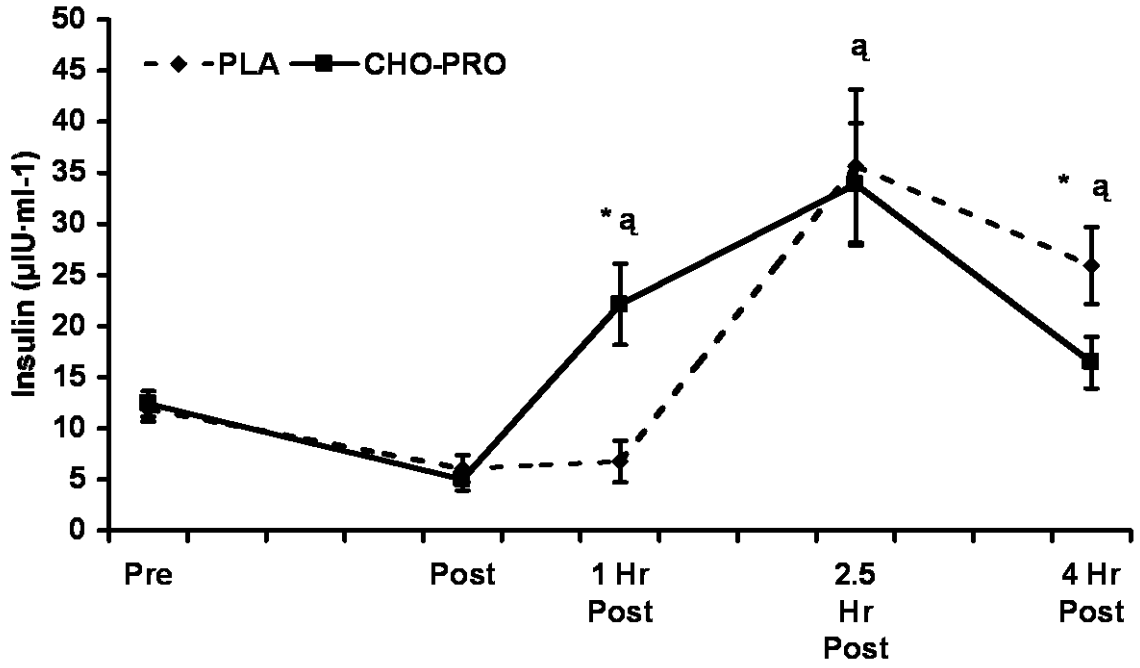

Figure 4. Serum insulin concentrations (mean ± SE). *, $p<0.05$, CHO-PRO vs. PL. †, $p<0.05$ vs. post (PLA); ‡, $p<0.05$ vs. post (CHO-PRO).

Muscle glycogen

Muscle glycogen significantly decreased in both trials (main effect for time, 141 ± 9 to 56 ± 8 mmol ·kg^{-1} wet wt.$^{-1}$ for pre and post-exercise, respectively). There were no differences between trials at any of the three time points (pre, 0 post, 4-hour post). Although glycogen concentration was still significantly lower at 4 hours post-exercise compared to pre-exercise, it was significantly higher than post-exercise values. There was no trial x time interaction indicating that the rate of glycogenolysis and the rate of glycogen synthesis were similar between trials (figure 5).

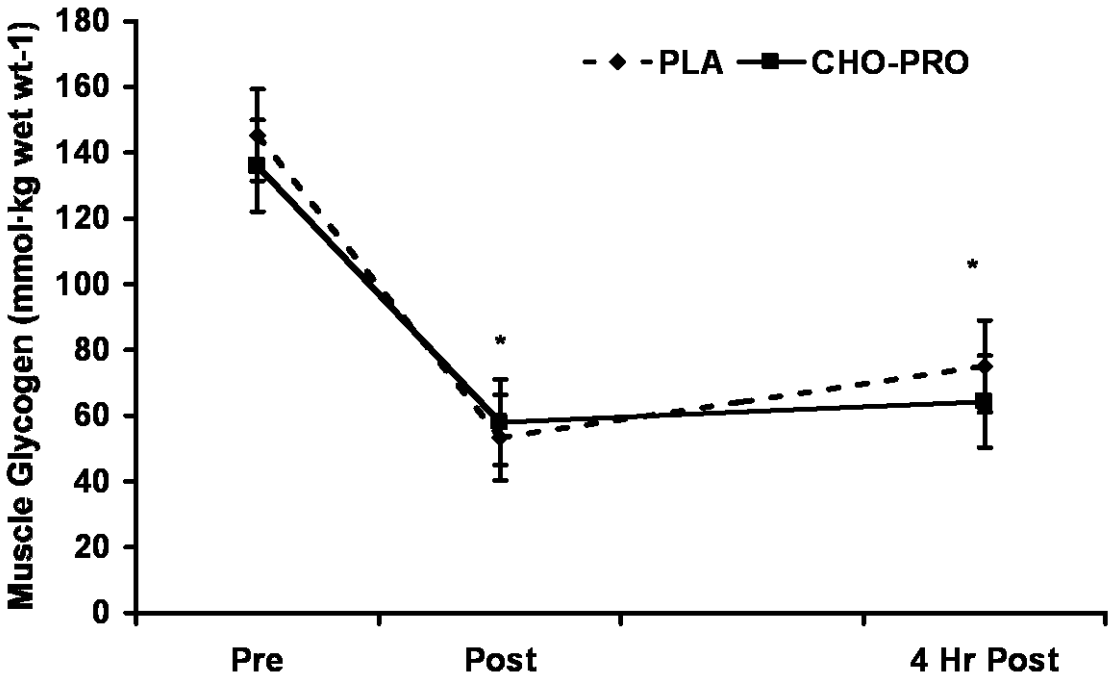

Figure 5. Muscle glycogen concentrations (mmol/kg wet wt) for pre, post and 4 hours post-exercise. *, $p<0.05$ vs. pre (main effect of time); †, $p<0.05$ vs. post (main effect of time).

Discussion:

With the development of reliable power meters that can be integrated into a rider's own bicycle, it is possible to conduct controlled cycling trials outside of the laboratory environment further extending the external validity of studies. In the present study we evaluated rates of muscle glycogen resynthesis during four hours of recovery from a 62 km road ride in response to two different recovery feeding strategies. The main finding in the current study demonstrated that the combination of a CHO-PRO beverage ingested 30 minutes post-exercise plus a standard meal two hours post-exercise did not increase muscle glycogen recovery more than ingesting the standard meal alone when subjects were fed before and during exercise.

In the current study the total CHO intake was 21% higher in the CHO-PRO vs. the PL trials during the 4-hour recovery period. The CHO-PRO drink contained 40g CHO, equaling ~0.29g $CHO \cdot kg^{-1} bw \cdot hr^{-1}$ (vs.0 for PL) and was ingested during the first two hours of recovery for the CHO-PRO trial. The meal received at two hours post-exercise contained 165g CHO equaling ~1.2 $g \cdot kg^{-1}$ $bw \cdot hr^{-1}$ for hours 2-4 of the recovery period. Therefore, the overall rate of CHO intake during the entire 4-hour recovery period was not equal (0.60 and 0.75 $g \cdot kg^{-1}$ $bw \cdot hr^{-1}$ for the PL and CHO-PRO trials, respectively.) The feeding protocol in the present investigation represents realistic approaches to pre, during and post-exercise feedings.

Similar rates of glycogen synthesis have been previously reported by Ivy et al. when glucose was

ingested at either 0.75 or 1.5g glucose·kg^{-1}bw·hr^{-1} during a 4-hour recovery period[15]. In addition, Blom et al. demonstrated comparable glycogen resynthesis rates when glucose was ingested at rates of 0.35 or 0.7 g glucose·kg^{-1}bw·hr^{-1} during six hours of recovery[4]. Despite subtle differences between trials for glucose and insulin response, the current data suggest that an additional CHO feeding shortly after exercise does not increase muscle glycogen resynthesis when a high carbohydrate meal is consumed approximately two hours later. The small differences in the glucose and insulin are likely the result of the timing and dose of post-exercise feedings across trials. Interestingly, even when diet is not isocaloric, muscle glycogen recovery was similar between trials.

The supplemental CHO immediately prior to and during the rides may have a potential interaction during the 4-hour recovery period. The majority of past research has compared rates of muscle glycogen resynthesis in subjects who were fasted prior to and during exercise. In the present study, glycogen resynthesis was evaluated under fed conditions that are more commonplace for endurance athletes. Our results are similar to the data of De Bock et al., who demonstrated lower rates of glycogen resynthesis when subjects were fed before and during 120 minutes of cycling vs. placebo. Rates of glycogen resynthesis (mean ± SD) during the 4-hour recovery period in their study were three times higher under fasted conditions (11.0 ± 7.8 vs. 32.9 ± 2.7 mmol· kg^{-1} dw ·hr^{-1} for the fed and fasted trials, respectively)[9].

Muscle glycogenolysis was similar between the two rides (92 ± 6 and 78 ± 14 mmol · kg^{-1} wet wt.$^{-1}$ ·2hr $^{-1}$ for PL and CHO-PRO trials, respectively) despite the fact that the exercise trials were completed outside of the typical well-controlled laboratory environment. These rates of glycogenolysis are similar to the rates reported by Ivy et al. (~ 84.7 mmol ·kg^{-1} wet wt.$^{-1}$ ·2hr $^{-1}$) in response to ~ 2.5 hours of cycling at 65-75% VO_2 peak with 1 minute intervals in the laboratory[13]. When the current data are taken together with previous research, field cycling trials appear to provide a reliable alternative method to laboratory based ergometer trials.

While previous investigations have clearly suggested nutritional protocols to increase rates of muscle glycogen resynthesis[4-6, 13-16, 20, 23, 25-27], it has also been shown that supplemental feeding during exercise may attenuate the demonstrated benefits of re-feeding strategies as they relate to glycogen recovery[12].

Conclusions:

The findings of this study, in combination with past laboratory data, demonstrate that a variety of post-exercise feeding strategies may accomplish the same magnitude of glycogen recovery.

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Lab Based Study 4. Caffeine and Carbohydrate Supplementation During Exercise When in Negative Energy Balance: Effects on Performance, Metabolism, and Salivary Cortisol

Dustin Slivka, Walter Hailes, John Cuddy, and Brent Ruby
Montana Center for Work Physiology and Exercise Metabolism
The University of Montana

Introduction:

Most laboratory-based investigations are conducted with subjects in a rested state after an overnight fast. However, this does not accurately represent the energy state of soldiers during extended military operations, peak physical training periods of athletes, or individuals involved in a weight loss strategy that includes diet and exercise. These individuals often find themselves in an extended state of negative energy balance. No studies have investigated the role of energy balance status on the effect of caffeine. However, a very low energy diet intervention for a period of one month has demonstrated that high habitual caffeine users reduced weight, fat mass, and waist circumference more than low caffeine users (Westerterp-Plantenga et al. 2005). Furthermore, it has been suggested that the energy state of the cell may affect caffeine mediated glycogen sparing (Chesley et al. 1998). Thus, by conducting this investigation with the subjects in negative energy balance a favorable environment for caffeine action may be created. The results from this study can then be applied to individuals who require optimal performance while in negative energy balance.

Caffeine taken in conjunction with carbohydrate has been shown to improve physical performance above that of carbohydrate alone (Kovacs et al. 1998). As a result, the sports supplement market is filled with caffeine containing carbohydrate gels and fluids and represents a commonplace nutritional strategy for athletes. Traditionally, caffeine was thought to increase fat oxidation (Ivy et al. 1979) and spare muscle glycogen (Ivy, Costill et al. 1979; Erickson et al. 1987; Graham et al. 1991). However, these traditional thoughts are currently being challenged. More recently, the co-ingestion of caffeine and carbohydrate has been shown to increase intestinal glucose absorption (Van Nieuwenhoven et al. 2000) and the rate of exogenous carbohydrate oxidation, thus increasing total carbohydrate oxidation (Yeo et al. 2005). Inconsistencies in the literature on the effects of caffeine and the effects of caffeine when taken along with carbohydrate on substrate use warrant further investigation.

Caffeine and exercise independently elevate cortisol levels (al'Absi et al. 2004). Increased cortisol levels stimulate lipolysis (Divertie et al. 1991; Djurhuus et al. 2002), which may help explain increased arterial FFA levels after the ingestion of caffeine (Graham et al. 2000). Exercise that does not challenge glucose homeostasis may not stimulate a cortisol response (Sung et al. 1990) since cortisol responds to falling blood glucose (Van Cauter et al. 1992). Thus, it is hypothesized that the intake of supplemental carbohydrate with caffeine during exercise may attenuate the cortisol response. In this way, cortisol can be used as a marker of overall stress and the demands placed on fuel homeostasis (Luger et al. 1987; Scavo et al. 1991; Laurent et al. 2000).

The purpose of this study was to determine performance and metabolic consequences of caffeine

ingestion with and without carbohydrate during a period of caloric restriction.

Materials and Methods:

Subjects

Eleven recreational male cyclists from the Missoula cycling community, aged 23 ± 3 y with a body mass of 74.1 ± 10.6 kg, a body composition of 13.0 ± 4.2% fat, a maximal oxygen consumption of (VO_2 max) of 59.5 ± 7.1 $ml \cdot kg^{-1} \cdot min^{-1}$, and a maximal power output (Wmax) of 294 ± 47 W took part in this study. The experimental procedures and potential risks involved with this study were explained and each subject signed a printed informed consent form. The study procedures were approved by the University Institutional Review Board (IRB) under Protocol #15-07.

Preliminary Testing

Maximum oxygen consumption was measured for each subject using a graded exercise protocol (starting at 95 watts and increasing 35 watts every three minutes) on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). Expired gases were collected during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and recorded at 15-second intervals. Maximum oxygen consumption was defined as the highest oxygen utilization recorded during a 15-second interval. Body density was determined using hydrodensitometry and corrected for estimated residual lung volume. Net underwater weights were recorded using a digital scale (Exertech, Dresbach, MN). Body density was then converted to body composition using the Siri equation (Siri 1993).

Experimental Design

Each subject completed four trials in a random order using a counter-balanced double blind crossover design. For two days before each trial, participants consumed a standardized diet consisting of one MRE military ration (1219 ± 134 Kcals/day, 164 ± 27 g carbohydrate, 47 ± 9 g fat, and 36 ± 10 g protein). Subjects were allowed to drink water *ab libitum* during the two days prior to each trial. Body weight was measured daily in order to track weight loss with the diet and exercise protocol. Cycle exercise was used due to its ability to target an easily sampled muscle (*vastus lateralis)*. Subjects cycled at 50% Wmax for two hours on the day before each trial (~ 24 hours prior to the experimental trials) in order to further achieve negative energy balance and to mimic activity patterns of extended military operations and training programs. Other than the lab based cycling exercise, only activities of daily living were allowed two days before each trial. This diet and exercise protocol ensured that each individual was in negative energy balance. The degree to which each individual was in negative energy balance varied due to differences in basal metabolic rate and daily activity energy expenditure. During each trial participants received either 1) placebo tablets and placebo drink (-CAF/-CHO), 2) placebo tablets and a 6% carbohydrate drink with the same flavor as the placebo drink (-CAF/+CHO), 3) caffeine tablets and placebo drink (+CAF/-CHO), or 4) caffeine tablets and 6% carbohydrate drink (+CAF/+CHO).

Protocol

On the day of the trial, participants arrived at the laboratory and saliva and muscle samples

(*vastus lateralis*) were obtained (Pre). Subjects then took either a caffeine tablet (200 mg, +CAF) or a dextrose (~0.18 g dextrose/tablet) placebo tablet (-CAF) at the beginning of and every 30 minutes while cycling for two hours at 50% Wmax. Subjects drank 500 ml of 6% carbohydrate (60 $g \cdot hr^{-1}$) beverage (+CHO) or similarly flavored placebo (-CHO) each 30 minutes during the two-hour ride. Participants were allowed to drink the beverage at a self-selected rate during each 30-minute period, but were required to drink the entire volume. Subjects ingested a total of 120 g of carbohydrate (60 $g \cdot hr^{-1}$) during the +CHO trials and/or 800 mg of caffeine (~5.3 $mg \cdot kg^{-1} \cdot hr^{-1}$) during the +CAF trials. This caffeine dosing protocol ensured a high level of circulating caffeine throughout the testing period. During the last 10 minutes of each hour Rating of Perceived Exertion (RPE, 6-20 scale) and expired gasses were collected. Gas was collected for five minutes with the last 3 minutes averaged and recorded to represent that sampling period. After the completion of the two-hour ride a second muscle biopsy was taken and a saliva sample was collected. Thirty minutes after the completion of the two-hour ride subjects performed a simulated 20 km time trial on the same Velotron cycle ergometer utilizing Racer Mate custom course software (RacerMate Inc., Seattle, WA). Participants were not familiarized with the performance trial, but each individual was familiar with the cycle ergometer from the previous tests and familiar with cycling in general from previous cycling experience. Average Watts produced and time to completion during the simulated flat 20 km time trail was recorded as the measure of performance. Heart rate was monitored (Polar USA, Lake Success, NY) throughout testing.

Biopsies

For each trial, biopsies were taken from the *vastus lateralis* muscle of the same leg in a randomized order using a 4-5 mm Bergstrom percutaneous muscle biopsy needle (Bergstrom 1962). Each successive biopsy on the same leg was obtained from a separate incision 2 cm proximal to the previous biopsy. After any excess blood, connective tissue, or fat were removed the tissue samples were immersed in liquid nitrogen and stored at -80°C for later analysis.

Muscle glycogen analysis

Muscle glycogen was analyzed using an enzymatic spectophotometric method. Samples were weighed (15.7 ± 0.6 mg wet weight) upon removal from an -80°C freezer and placed in 0.5 ml, 2N HCl solution. The sample solutions were weighed, incubated for two hours at 100°C in an oven, then re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5ml of 0.67 NaOH was added. A volume of this muscle extract (20µl) was added to 1 ml of Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read on a spectrophotometer at 340nm. Muscle glycogen was then calculated using the extinction co-efficient of NADH. Muscle glycogen concentrations are expressed in mmol $\cdot$ kg^{-1} wet weight of muscle tissue. The coefficient of variation for duplicate samples was 5.3 ± 5.9%.

Salivary Cortisol

Saliva was collected (~3 ml) by passive drool and frozen at -30º C. Salivary cortisol was measured using a competitive immunoassay on a plate reader (Model 680 XR, Bio-Rad, Hercules, CA) at 450 nm in accordance with the manufacture's protocol (Salimetrics, State College, PA). The coefficient of variation for duplicate samples was 4.3 ± 3.7%.

Statistics

Glycogen utilization, body weight, and cortisol response between trials over time (pre vs. post) were compared using two-way repeated-measures ANOVAs. Fat oxidation, carbohydrate oxidation, RER, and RPE between trials over time (hour 1 vs. hour 2) were compared using two-way repeated-measures ANOVAs. Time trial measures were analyzed using one-way repeated measures ANOVAs. In the event of a significant F ratio the false detection rate method (Benjamini et al. 1995) was applied (R 2007) to locate differences and correct for multiple comparisons. All ANOVAs were performed using SPSS for windows Version 9 (Chicago, IL). A probability of type I error less than 5% was considered significant ($p<0.05$). All data are reported as means ± SD.

Results:

Body weight changes were monitored to quantify the state of energy balance of each subject. Subjects lost 1.11 ± 0.83 kg ($p<0.05$) during the two days prior to each trial. During each exercise trial 2 liters (2 kg) of fluid was ingested resulting in a 0.33 ± 0.42 kg ($p<0.05$) weight gain. There was no difference ($p>0.05$) between trials.

Muscle Glycogen

The rate of muscle glycogenolysis during the 2-hour exercise was similar for all trials ($p>0.05$). Muscle glycogen decreased 43% during the 2-hour exercise bout regardless of trial ($p<0.05$, Figure 1).

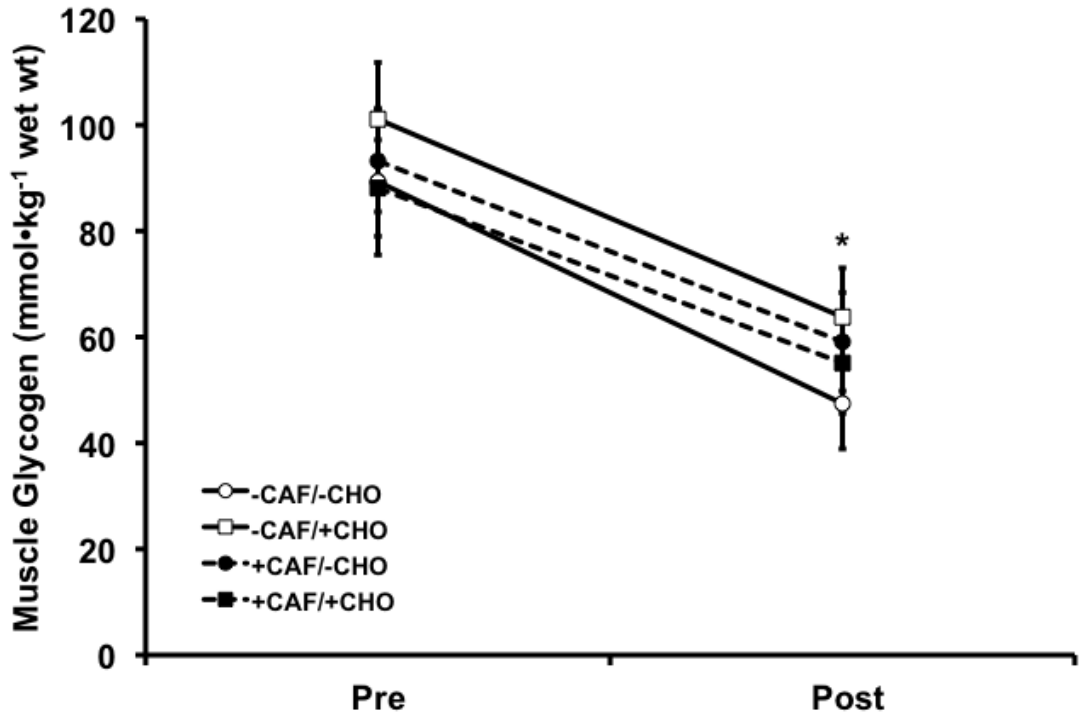

Figure 1. Changes in muscle glycogen content following 2-hours of cycling exercise (Pre-Post) in response to caffeine and carbohydrate feedings. * $p<0.05$ for all trials from Pre.

Fat Oxidation

There was no difference in fat oxidation between -CAF/-CHO (0.737 ± 0.223 g·min^{-1}) and +CAF/-CHO (0.779 ± 0.181 g·min^{-1}, $p<0.05$). There was a trend for increased fat oxidation in the +CAF/+CHO (0.682 ± 0.173 g·min^{-1}) trial than in the -CAF/+CHO (0.622 ± 0.234 g·min^{-1}, $p=0.069$) trial. Additionally, fat oxidation was higher during the -CAF/-CHO trial and the +CAF/-CHO trial than in the -CAF/+CHO trial ($p<0.05$). No differences were noted between the first hour of exercise and the second hour of exercise ($p>0.05$).

Carbohydrate Oxidation

There was no difference in carbohydrate oxidation be between -CAF/-CHO (1.028 ± 0.328 g·min^{-1}) and +CAF/-CHO (0.985 ± 0.122 g·min^{-1}, $p<0.05$). There was a trend for decreased carbohydrate oxidation in the +CAF/+CHO (1.195 ± 0.214 g·min^{-1}) trial than in the -CAF/+CHO (1.362 ± 0.244 g·min^{-1}, $p=0.069$). Additionally, carbohydrate oxidation was lower during the -CAF/-CHO trial and the +CAF/-CHO trial than in the -CAF/+CHO trial ($p<0.05$). No differences were noted between the first hour of exercise and the second hour of exercise ($p>0.05$).

RER

The intake of +CHO during exercise increased whole body respiratory exchange ratio (RER). RER was higher in the -CAF/+CHO trial than the -CAF/-CHO trial and in the +CAF/+CHO trial over the +CAF/-CHO trial ($p<0.05$). The +CAF/+CHO trial produced a lower RER than that of the -CAF/+CHO trial ($p<0.05$). However, substrate use patterns were similar between +CAF/-CHO and -CAF/-CHO trials ($p>0.05$, Figure 2). No differences were noted between the first hour of exercise and the second hour of exercise ($p>0.05$).

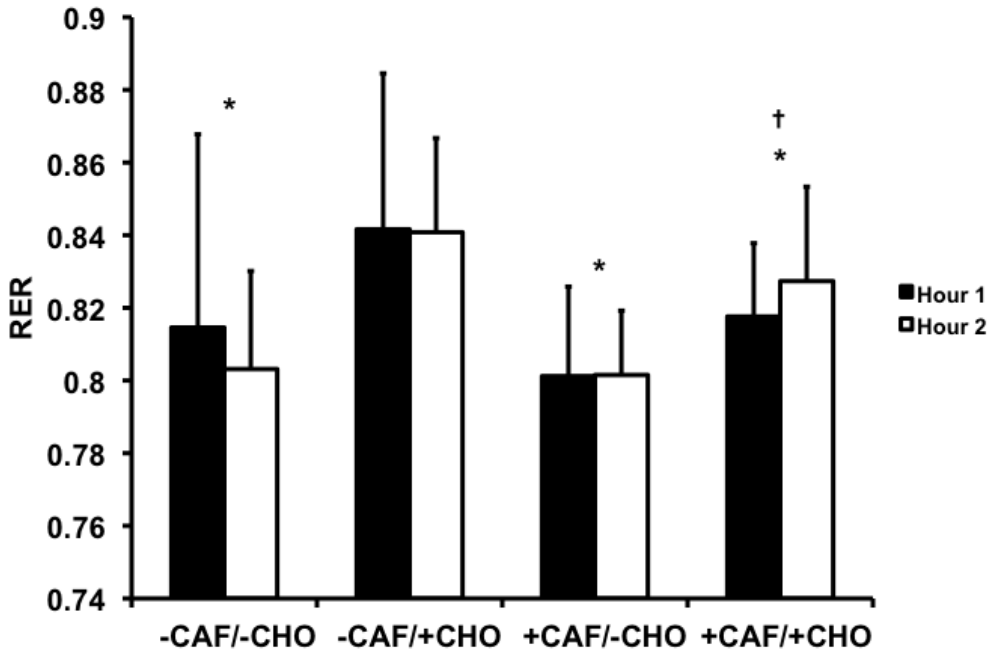

Figure 2. RER during 2-hours of cycling exercise in response to caffeine and carbohydrate

feedings. * p<0.05 compared to –CAF/+CHO, † p<0.05 compared to +CAF/-CHO.

RPE

Rating of perceived exertion (RPE) was lower during the first hour of exercise (12 ± 2) than in the second hour of exercise (13 ± 2, p<0.05). No differences in RPE were noted between the trials (p>0.05).

Heart Rate

There was no difference in the average heart rate response during the two-hour ride between any of the trials (-CAF/-CHO, 139 ± 9 bpm; -CAF/+CHO, 137 ± 7 bpm; +CAF/-CHO, 137 ± 7 bpm; +CAF/+CHO, 138 ± 8 bpm; p>0.05). Each trial was conducted at the same relative workload (50% Wmax).

Cortisol

The cortisol response to exercise was greater in +CAF/-CHO than in all other trials (p<0.05, Figure 3). Furthermore, no correlations between cortisol response and substrate use were found.

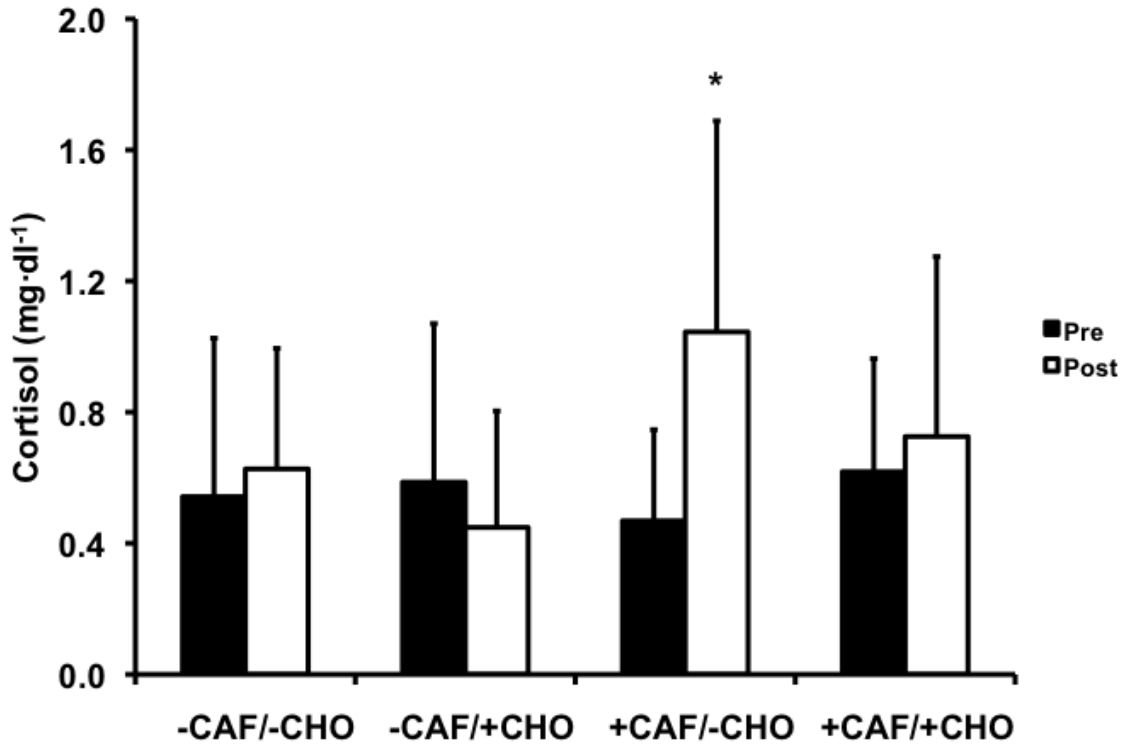

Figure 3. Changes in salivary cortisol following 2-hours of cycling exercise (Pre-Post) in response to caffeine and carbohydrate feedings. * p<0.05 from all other trials (group x time interaction).

Performance

Simulated 20 km time trial performance (average watts) was greater in -CAF/+CHO than in -CAF/-CHO (p<0.05, figure 4). There were no other differences in average watts between trials.

Additionally, The time to complete the 20 km time trial was not different ($p>0.05$) between any of the trials (40.5 ± 7.4, 37.2 ± 4.2, 38.7 ± 7.1, and 37.3 ± 4.9 minutes for the -CAF/-CHO, -CAF/+CHO, +CAF/-CHO, and +CAF/+CHO trials, respectively).

Heart rate during the 20km time trial was not different between any of the trials (151 ± 18, 162 ± 12, 153 ± 24, and 162 ± 20 bpm for the -CAF/-CHO, -CAF/+CHO, +CAF/-CHO, and +CAF/+CHO trials, respectively).

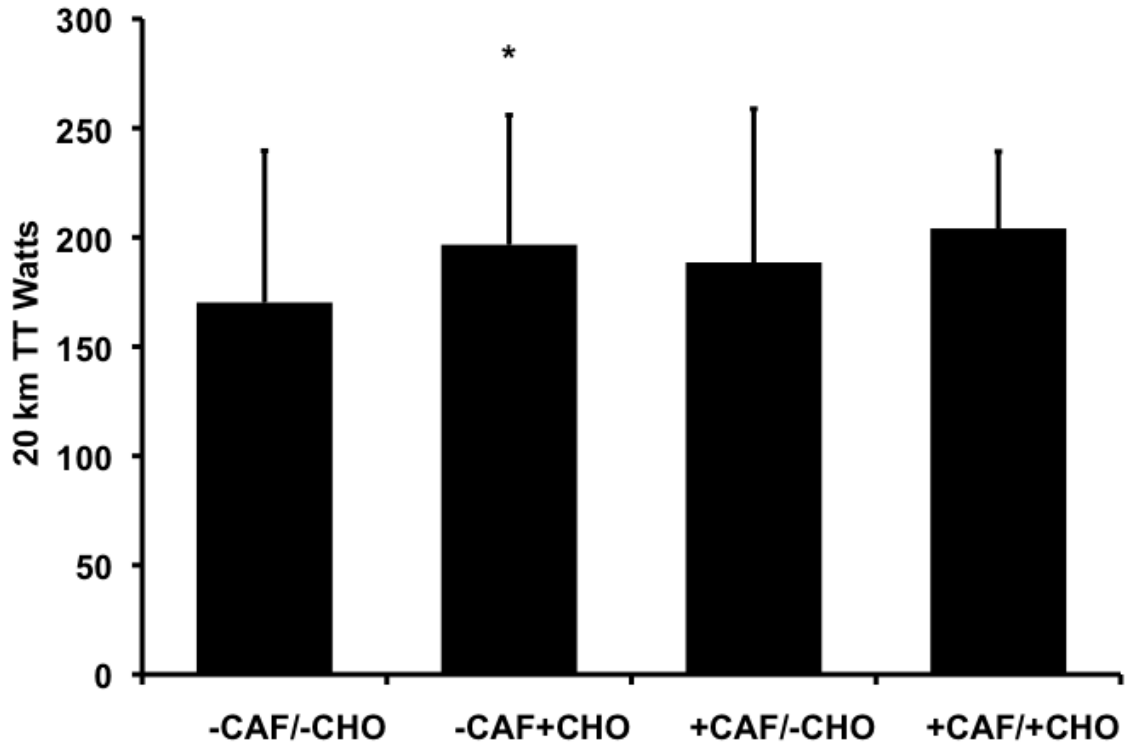

Figure 4. Average Watts during a simulated 20 km cycling time trial in response to caffeine and carbohydrate feedings. * $p<0.05$ compared to -CAF/-CHO.

Discussion:

The aim of the current study was to determine the effects of caffeine when consumed with and without carbohydrate during exercise while in negative energy balance. The protocol to stimulate a state of negative energy balance yielded a 1.11 ± 0.83 kg weight loss over the course of two days, supporting that negative energy balance was achieved. The observed weight loss from the current diet and exercise protocol is likely a combination of reduced substrate stores and reduced water associated with those stores. The current data demonstrate that the ingestion of caffeine with carbohydrate may have important metabolic, hormonal, and psychological benefits over the ingestion of carbohydrate or caffeine alone.

Substrate Use

Early studies that investigated the effects of caffeine on glycogen reported a glycogen sparing

effect (Ivy, Costill et al. 1979; Erickson, Schwarzkopf et al. 1987; Graham and Spriet 1991). However, the current project and other recent investigations have failed to confirm this finding (Jackman et al. 1996; Chesley, Howlett et al. 1998; Graham, Helge et al. 2000; Greer et al. 2000; Laurent, Schneider et al. 2000). Furthermore, mechanism based studies have shown an increase in muscle glycogen phosphorylase (Chesley, Howlett et al. 1998) and increased glycogen breakdown in fast-oxidative fibers with caffeine supplementation (Vergauwen et al. 1997). The current study did not find any difference in muscle glycogen use with caffeine, carbohydrate, or a combination of the two.

Previous reports regarding the effects of caffeine indicate either a decrease in RER (Costill et al. 1978; Ivy, Costill et al. 1979; Bangsbo et al. 1992) or no difference in RER (Wells et al. 1985; Erickson, Schwarzkopf et al. 1987; Tarnopolsky et al. 1989; Vandenberghe et al. 1996; Engels et al. 1999; Turley et al. 2007). The reason for differing results is not apparent, as it does not seem to be related to training status or exercise intensity. In the current study exercise RER was not affected by caffeine alone. However, when caffeine was taken in conjunction with carbohydrate (+CAF/+CHO) a lower RER was observed over that of carbohydrate alone (-CAF/+CHO) indicating a substrate use shift towards increased fat oxidation and decreased carbohydrate oxidation. Other investigations have shown no change in RER when caffeine was added to carbohydrate supplementation (Gaesser et al. 1985; Erickson, Schwarzkopf et al. 1987; Vandenberghe, Gillis et al. 1996; Jacobson et al. 2001). When the current data are combined with previous reports, nutrient availability may be an important factor in understanding the underlying effects and mechanism(s) of caffeine on substrate use. While protein oxidation was not measured as part of this investigation, it may be increased with reduced energy/carbohydrate intake (Tarnopolsky 2004). Thus, the -CAF/-CHO and +CAF/-CHO conditions may have yielded higher rates of protein oxidation, which are not reflected in the non-protein RER values reported here.

Cortisol

The current study demonstrates that the ingestion of caffeine during exercise increases salivary cortisol when it is not taken with carbohydrate. The increase in cortisol was abolished when carbohydrate was co-ingested with caffeine. This finding may not seem surprising given that cortisol responds to overall stress and the demands placed on fuel homeostasis (Luger, Deuster et al. 1987; Scavo, Barletta et al. 1991; Laurent, Schneider et al. 2000). However, the physiological demands were the same for the -CAF/-CHO trial, which did not show a significant increase in cortisol, thus indicating hypercortisolemia with caffeine supplementation can be reduced if the caffeine is co-ingested with carbohydrate.

RPE

Rating of perceived exertion was not statistically different between any of the trials, but it did increase from hour 1 to hour 2. A recent meta-analysis (Doherty et al. 2005) revealed that caffeine appears to reduce RPE during exercise over placebo by an average of 5.6%. The results of the current study show a non-significant 6.0% reduction in RPE with the ingestion of caffeine and no carbohydrate (-CAF/-CHO vs. +CAF/-CHO) and an 8.0% reduction when caffeine was ingested with carbohydrate (-CAF/+CHO vs. +CAF/+CHO). In qualitative terms, the addition of caffeine and carbohydrate to a placebo would take an individual from a "somewhat hard" intensity to a "fairly light" intensity. Thus, while the current results are not statistically

significant, they are in agreement with previous research and support the notion that reduced perceived exertion by subjects may account for some of the previously reported ergogenic benefits of caffeine ingestion.

Performance Trial

The ergogenic benefits of caffeine (Graham 2001) and carbohydrate (Jeukendrup 2004) on endurance performance are well established. The current study did show a performance benefit during a simulated 20 km time trial when carbohydrate only (-CAF/+CHO) was ingested over no caffeine or carbohydrate (-CAF/-CHO). However, the current study failed to show a benefit in performance when only caffeine was ingested (+CAF/-CHO vs. -CAF/-CHO). Furthermore, the performance enhancing effect of carbohydrate seemed to be negated by caffeine. When performance data are considered with the substrate use data, it appears that the shift in fuel selection toward more fat and less carbohydrate that occurs with caffeine supplementation when carbohydrate is co-ingested (-CAF/+CHO vs. +CAF/+CHO) may limit exercise intensity. It is therefore possible that performance may be increased under these dietary circumstances if the duration of the performance trial was longer and intensity was lower (a circumstance where increased fat metabolism would be beneficial). The relatively short nature of the performance trial, the exercise bout prior to the performance trial, timing of the performance trial, and the state of energy balance during the current investigation may play a role in the lack of ergogenic effect with caffeine shown here.

Significance and Conclusions

The unique aspect of this project was that the participants were in negative energy balance for two days prior to and during each trial. This aspect extends the external validity of this study to: 1) extended military operations where load carriage limits the amount of food that is available/consumed, 2) periods of peak physical training or competition when it is difficult to match energy intake with energy expenditure, 3) weight loss strategies that include diet and exercise, and 4) any circumstance where energy balance is not maintained. The impact(s) that energy state had on the results is unknown, as this study did not directly compare caffeine and carbohydrate effects during positive and negative energy balance. Further research is needed to determine the impact of energy status on the effects of caffeine and caffeine co-ingested with carbohydrate.

The current data demonstrate that during negative energy balance, the inclusion of caffeine with carbohydrate supplementation lowers RER, shifting metabolism towards increased fat and decreased carbohydrate oxidation. This shift in metabolism was not apparent when caffeine was ingested without carbohydrate. While the current study did not show an increase in 20 km cycling time trial performance when caffeine was co-ingested with carbohydrate over the ingestion of carbohydrate alone, the observed shifts in substrate use toward fat utilization may be beneficial during longer duration activities. Additionally, the increased cortisol response demonstrated with caffeine supplementation was abolished when carbohydrate was co-ingested.

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Lab Based Study 5. Effect of post-exercise environmental temperature on glycogen resynthesis

M. Naperalsky, D. Slivka, and B.C. Ruby
Montana Center for Work Physiology and Exercise Metabolism
The University of Montana

Introduction:

During long durations of physical work, the body's stored fuel can become greatly depleted, requiring sufficient recovery to provide adequate energy for future activity. Acute exercise in the heat has shown a greater use of carbohydrates (glycogen) compared to a cooler environment, specifically targeting endogenous stores independent of exogenous supply. That is, the body utilizes already-stored glycogen in preference over supplied carbohydrate.

This preference makes adequate glycogen synthesis of high importance to fuel future work. Muscle glycogen recovery has been shown to be influence by the amount, timing, and composition of post-exercise meals, as well as enzymatic factors relating to glucose storage. Although multiple factors may hasten or impair glycogen recovery, the effects of ambient and core body temperature on glycogen recovery have not been addressed.

The purpose of this study was to compare hot and thermoneutral environments and the impact they may exert on glycogen recovery following exercise. These results will allow examination of environmental factors affecting physical recovery, which has direct application to future performance.

The results of these data may be applicable for a variety of populations that require both strenuous work and recovery in the heat. These data may allow for recommendations aimed to enhance recovery from exercise.

Methods:

Subjects
Nine (n = 9) male participants from the Missoula community served as subjects. These individuals were recruited because of their familiarity with cycling or strenuous exercise. Most had prior experience with completing challenging rides at a moderate percentage of their maximal work rates. Prior to data collection, the protocol was approved by the institutional review board (Protocol # 11-08).

Body Composition
Body density was determined using hydrodensitometry and corrected for estimated residual lung volume. Net underwater weights were recorded using a digital scale (Exertech, Dresbach, MN). Body density was then converted to body composition using the Siri equation.

Exercise capacity
Maximum oxygen consumption (VO_2max) and workload associated with VO_2max was measured

for each subject using a graded exercise protocol (starting at 95 watts and increasing 35 watts every three minutes) on an electronically braked cycle ergometer trainer (Velotron, RacerMate Inc., Seattle, WA). Participants rode to volitional fatigue. Maximum workload was calculated as the highest completed stage (in watts) + the proportion of time multiplied by the 35 watt stage increment. Expired gases were collected during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and recorded at 15-second intervals. During each stage, the average expired gas values for the last 1 minute was recorded for the calculation of RER and sub-maximal substrate oxidation.

Exercise Trials

Subjects completed two, 1-hour cycle rides at 60% of VO_{2peak} in a heated environmental chamber at an ambient temperature of approximately 32ºC using an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). Subjects were allowed water *ad libitum* up to 500 mL during the 1-hour cycle rides. Following each ride subjects rested for a 4-hour recovery period in either the same heat chamber (32˚C) or in a separate area with an ambient temperature of approximately 22ºC. Core temperature was continually measured during exercise and recovery using a rectal thermistor, and recorded at 1-minute intervals using a digital data logger (OM-3000, OMEGA Engineering, Inc., Stamford, CT). During recovery subjects were fed an oral dextrose solution (1.8 g • kg-1 BW) immediately after exercise, and again two hours later.

Expired Gases

Expired gases were collected for two 5-minute periods during the recovery period, at 105-110 minutes and 225-230 minutes post-exercise, corresponding to 0-2 hours and 2-4 hours of recovery. Samples were measured using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT), with 30-second samples averaged over 2.5 minutes to calculate respiratory exchange ratio and determine fuel oxidation.

Biopsies

Biopsies were taken from the *vastus lateralis* muscle using a 4-5 mm Bergstrom percutaneous muscle biopsy needle (Bergstrom 1962). One leg was used for 3 biopsies for the first trial, alternating to the other leg for the 3 biopsies of the second trial. Each successive biopsy on the same leg was obtained from a separate incision 2 cm proximal to the previous biopsy. After any excess blood, connective tissue, or fat were removed and tissue samples were immersed in liquid nitrogen and stored at -80°C for later analysis. Biopsies were obtained immediately post-exercise, 2 hours post-exercise, and 4 hours post-exercise for analysis of muscle glycogen. (see below).

Glycogen

Muscle glycogen was analyzed using an enzymatic spectophotometric method. Samples were weighed upon removal from an -80°C freezer and placed in 0.5 ml, 2N HCl solution. The sample solutions were weighed, incubated for two hours at 100°C in an oven, then re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5ml of 0.67 NaOH was added. A volume of this muscle extract (20μl) was added to 1 ml of Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read on a spectrophotometer at 340nm. Muscle glycogen was then calculated using the extinction co-efficient of NADH. Muscle

glycogen concentrations are expressed in mmol · kg^{-1} wet weight of muscle tissue.

Statistics

Differences in muscle glycogen, core temperature, and RER were compared between treatments and by time (Post, 2-hrs, 4-hrs post-exercise) using two-way repeated-measures ANOVA's. Difference in ambient temperature for recovery environments was compared using a two-tailed paired t-test, using Excel 2007 (Microsoft Corp., Redmond, WA). All ANOVAs were performed using SPSS for Windows version 9 (Chicago, IL). A probability of type I error less than 5% was considered significant ($p<0.05$). All data are reported as means ± SE (Table 1). In the event of a significant F ratio the false detection rate method (Benjamini and Hochberg 1995) was applied (R Development Core Team 2007) to locate differences and correct for multiple comparisons.

Results:

Table 1. Subject descriptive data

	Mean	SE
Age (yrs)	24.11	± 0.98
Height (cm)	178.44	± 2.14
Body Weight (kg)	79.35	± 2.69
Body Composition (% fat)	16.8%	± 0.01
VO_2max (L/min)	4.29	± 0.29
Max Power (watts)	293.00	± 22.3
Average Ride Watts	173.24	± 11.6

Muscle Glycogen

Muscle glycogen synthesis was lower during the second half of recovery (hours 2 to 4) in the hot environment. Muscle glycogen increased in both trials from Post to 2-hours Post. There was no difference in muscle glycogen between hot and thermoneutral conditions at Post or 2-hours Post exercise. See Figure 1.

(mmol·kg^{-1}

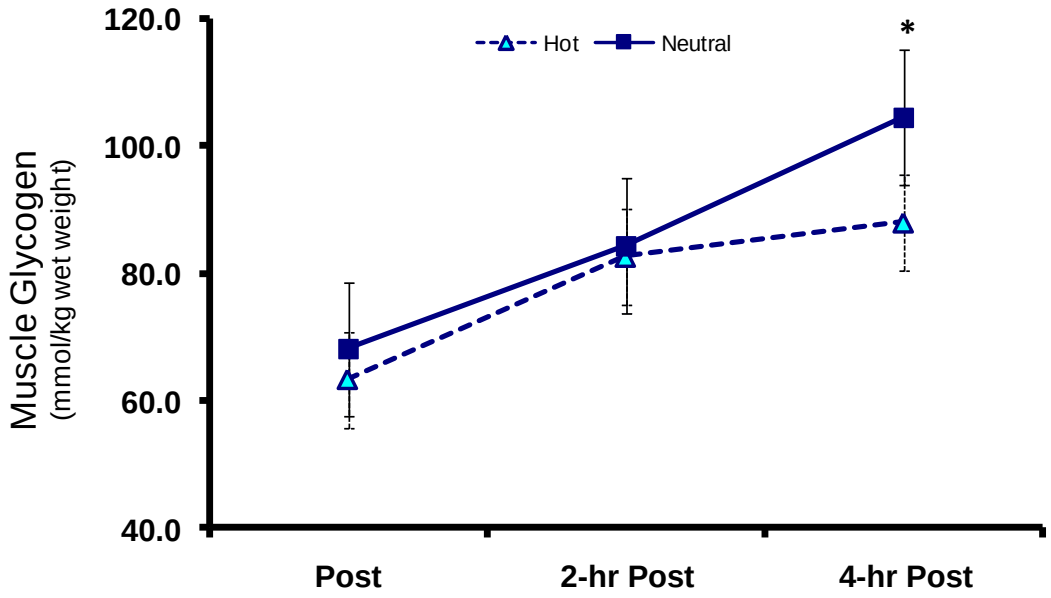

Figure 1. Muscle glycogen during recovery, at Post, 2 hours and 4 hours post-exercise. * $p<0.05$ from 2-hrs to 4-hrs Post in heat (time x trial interaction).

Core temperature
Core temperature was higher during recovery in the hot environment compared to the thermoneutral recovery. There was no difference in core temperatures between 0-2 hours and 2-4 hours of recovery for either trial. See Figure 2.

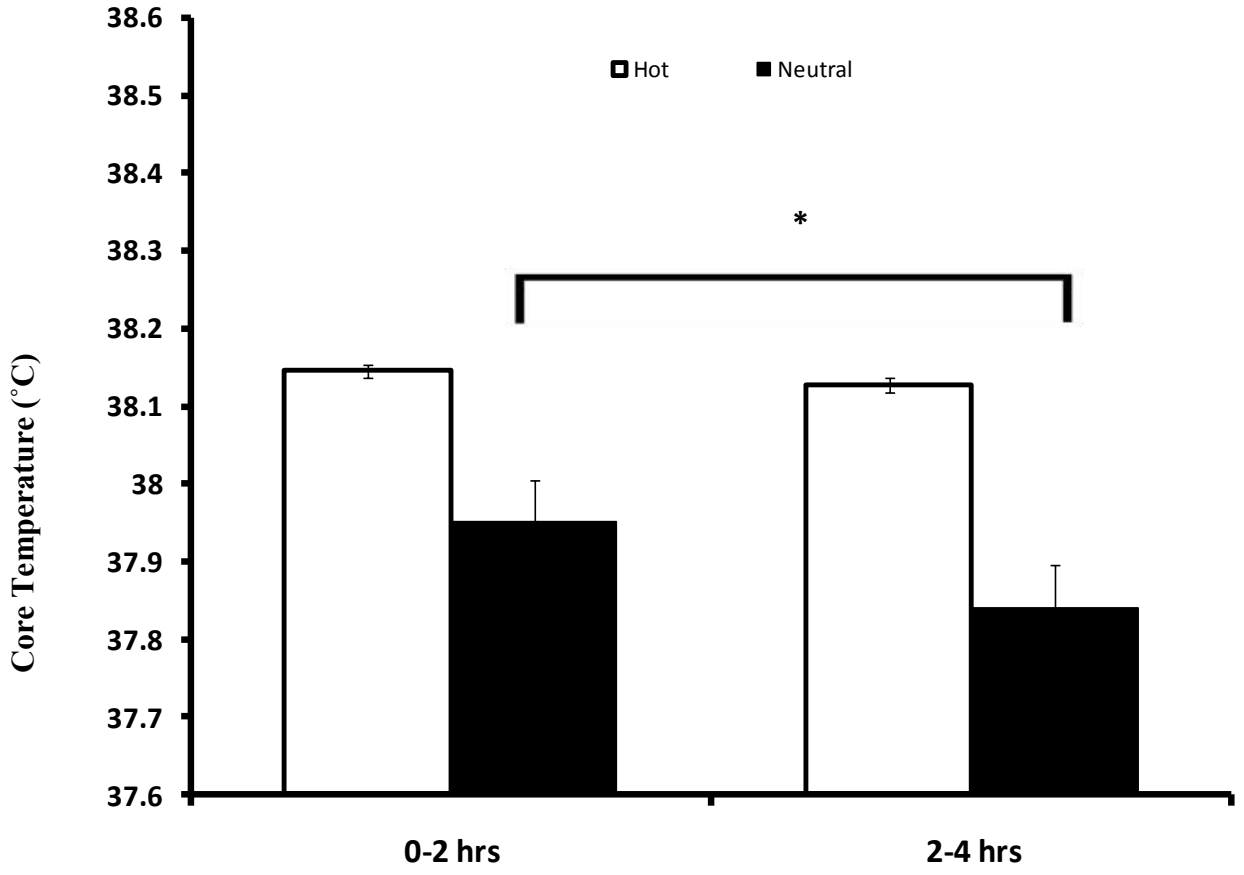

Figure 2. Core temperature during recovery, averaged over the first 2 hours and second 2 hours. * $p<0.05$ Neutral from hot (main effect for trial).

Ambient temperature
Ambient temperature for the hot recovery environment was warmer than the thermoneutral recovery environment ($p<0.05$).

Whole body RER
RER for 2-4 hours was higher ($p<0.05$) than 0-2 hours of recovery for both trials. There was no difference in RER between hot and thermoneutral trials. See Figure 3.

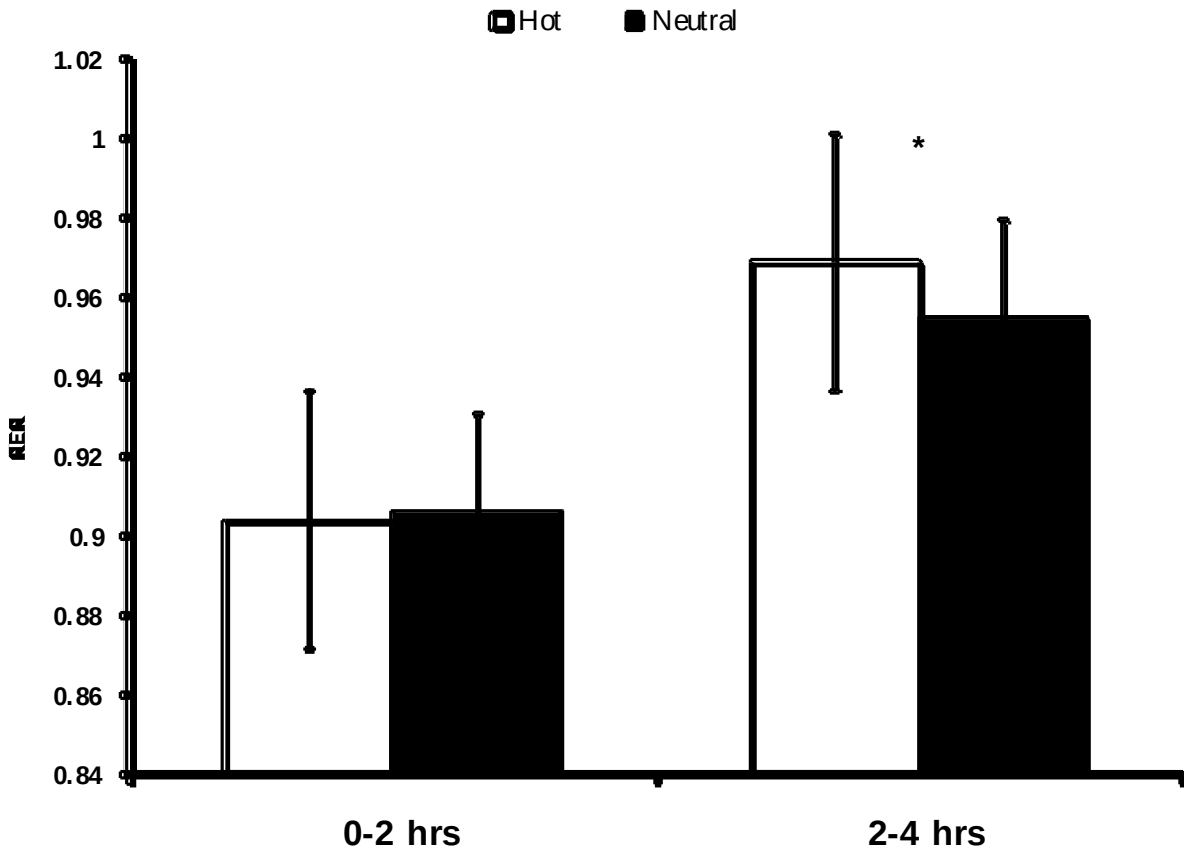

Figure 3. RER at 0-2 hours and 2-4 hours of recovery. *p<0.05 from 0-2 hours.

Discussion:

The main findings associated with this study were that a hot environment impairs muscle glycogen synthesis following exercise in the heat during 4 hours of recovery compared to a thermoneutral environment.

During the 4-hour period, muscle glycogen increased (synthesis) at all three time points during the recovery in a thermoneutral environment, from 0 to 2 hours and from 2 to 4 hours. In the hot environment, glycogen resynthesis increased from 0 to 2 hours but did not increase further from 2 to 4 hours.

Core temperature was significantly higher during the hot trial ($p<0.05$) compared to the thermoneutral trial. There was no difference, however, between the first and second halves of the recovery period for either environment, which may be a function of general exercise recovery and the elevated core temperature associated with activity.

Expired gas values used to calculate whole-body RER showed altered substrate utilization between 0-2 hours and 2-4 hours of recovery. Both trials showed consistent values for each

interval, with no effect for time. Both neutral and hot environments showed increased carbohydrate metabolism in the second half of recovery as the body attempted to synthesize glycogen. These data suggest a slight delay in gastric emptying due to elevated core temperature. Moreover, recovery in a hotter environment may further impair CHO delivery from the gut thereby reducing glycogen recovery within the skeletal muscle.

Impaired glycogen synthesis during recovery in a hot environment may be caused by altered blood flow throughout the body. As the body works to maintain a steady core temperature, blood may be directed toward the skin for thermoregulation instead of toward the gut for the processes of digestion and absorption. A higher RER during the second half of recovery may indicate a delayed emptying of the stomach and/or metabolism of ingested carbohydrate to be delivered to recovering muscle. Both core temperature and RER data suggest that thermoregulation may take precedence over glycogen synthesis following exercise in the heat. This is especially true if the recovery period occurs in a hotter environment.

Conclusions:

The data suggest that increased ambient temperature directly affects the amount of glycogen storage for muscle recovery. This may impair ensuing bouts of physical work that would demand adequate fuel and maximum recovery. The environment of recovery should be considered when recommending optimal procedures for greatest efficacy.

These data have direct relevance to military operations in that if glycogen is compromised and rapid recovery for subsequent exercises and/or missions is imperative, recovery in a cooler climate will enhance skeletal muscle glycogen resynthesis compared to recovery in a hotter climate.

Lab Based Study 6. Effects of fabric type on core temperature and exercise performance in the heat

W. Hailes, J. Cuddy, J. Ives, D. Slivka, B. Ruby
Montana Center for Work Physiology and Exercise Metabolism
The University of Montana

Supplemental Funding: THY Enterprises

Introduction:

Training to become and perform as a United States Air Force Special Operations warfighter requires specialized physical work and mental ability. Performing activities in hot environments produces an additional stress on individuals and requires additional effort to maintain thermal homeostasis. Attempting to maintain core body temperature at the typical 37ºC while working in the heat requires adjustments to normal body functions. Working muscles require increased blood flow for nutrient delivery and waste removal. Maintenance of core temperature requires increased blood flow to the skin to maximize heat dissipation to the external environment (Kenney, 1992). Therefore exercising in hot environments requires either that cardiac output increases for a given workload to supply adequate blood to the working muscles and the skin for heat loss or cardiac output is maintained and work rate decreases (Harrison, 1986, Wilmore 2004). In addition to changes in blood flow kinetics, sweat rate increases in an attempt to increase evaporative cooling. As sweat is lost, plasma volume decreases requiring an additional increase in heart rate to maintain adequate blood pressure (Wilmore 2004). Maintenance of normal plasma volume and therefore cardiac output is the primary reason maintaining adequate hydration during activities in hot environments is important. Since a specific level of performance is required for many activities/operations, a reduction in work rate is often not possible. Therefore, if the heat stress is intense enough individuals can experience dangerous increases in core temperature and severe dehydration while attempting to manage the stress of the operation and the heat.

In an attempt to minimize the effects of the heat many different clothing styles, fabrics, designs and colors have been used by individuals required to perform in hot environments, including soldiers and athletes. For example, in environments with intense solar radiation light colors that reflect radiation help individuals remain cooler compared to dark colors that absorb much of the radiation. Fabrics that promote the body's natural cooling mechanism, sweating, by wicking moisture from the skin to the environment can potentially help reduce heat stress compared to fabrics that do not allow evaporative cooling. Fabrics that allow increased airflow over the skin surface also have the potential to increase cooling through increased convective heat loss. The purpose of this study is to examine the effects of technical fabrics on measurements of body temperature and performance during exercise in a heated environment. The goal of the study will be to determine which fabric reduces the overall heat stress load on the individual.

Methods:

Twelve recreationally trained individuals from the Missoula community (7 males, 5 females, 23±2.4yrs, 74.3±15.0kg, 178.6±9.4cm, 57.6±4.4ml·kg^{-1}·min^{-1}) volunteered and gave informed consent to participate in this study which was approved by The University of Montana Institutional Review Board (Protocol # 13-07). Each subject completed a maximum exercise capacity test (Bruce protocol) on a treadmill (while expiratory gas was collected using a calibrated metabolic cart (Parvomedics, Salt Lake City, UT) and peak oxygen consumption (VO_{2peak}) was recorded. Following the maximal exercise testing each subject completed four trials in the heat separated by at least seven days to minimize the effects of heat acclimatization. Each trial consisted of 45 minutes of walking followed by a one mile time trial run completed as quickly as possible. Participants did not leave the hot environment (39.3 ± 0.1 degree C) until completion of the entire trial. Every participant completed the 45 minutes of walking at approximately 35% of their VO_{2peak}. Using the American College of Sports Medicine prediction equation (below), participants walked at a constant 5.6 kph with varying degrees of incline to obtain the desired workload.

Walking (<6.4kph): VO_2 (mL·kg^{-1}·min^{-1}) = 0.1 (m·s^{-1}) + 1.8(m·s^{-1}) (fractional grade) +3.5

A total of four trails were completed in a randomized order with subjects wearing the same clothing for every trial except for the four different short sleeved shirts (Table 1).

Table 1: A list of the four shirts that were tested and the fabric type of each shirt.

Shirt	Shirt Fabric
Air Force PT (PT)	100% Polyester
THY (THY)	96% Polypropylene, 4% Other fibers
Under Armor (UA)	80% Polyester, 20% Elastane
Air Force Undershirt (US)	100% Cotton

Core temperature, heart rate, ambient temperature and wet bulb temperature were collected every minute and Rating of Perceived Exertion (RPE) was collected every five minutes during the 45 minutes of walking. Core temperature and heart rate were combined to determine the physiological strain index (PSI). During the one mile time trial, heart rate, ambient temperature and wet bulb temperature were collected every minute. Nude body weight was recorded prior to and following each trial to estimate sweat loss.

Results:

There were no significant difference in core temperature, heart rate, PSI, ambient temperature, wet bulb temperature or RPE between trials for the 45 minutes of walking and no difference in change in body weight(Δ weight) from pre to post exercise(Table 2).

Table 2. Measured variables during the 45 minutes of exercise and Δ body weight

	THY	Under Armor	Air Force PT	Air Force Undershirt
Ambient Temp (ºC)	39.1 ± 0.7	39.4 ± 0.6	39.2 ± 0.6	39.1 ± 0.7
Wet Bulb Temp (ºC)	20.6 ± 1.6	20.5 ± 1.7	20.5 ± 3.7	20.3 ± 2.7
Heart Rate	118.9 ± 14.2	123.0 ± 16.9	122.5 ± 15.3	121.8 ± 13.9
Core Temp(ºC)	38.0 ± 0.4	38.2 ± 0.5	38.1 ± 0.5	38.0 ± 0.4
RPE	11 ± 2	11 ± 2	11 ± 2	10 ± 2
PSI	2.4 ± 1.7	2.5 ± 2.1	2.4 ± 1.6	2.1 ± 1.7
Δ weight (kg)	2.0 ± 0.8	1.9 ± 0.6	2.2 ± 0.7	2.2 ± 0.7

There were no significant difference in 1 mile run time, heart rate, ambient temperature or wet bulb temperature between each shirt during the performance trial (Table 3).

Table 3. Measured variables during the performance trial

	THY	Under Armor	Air Force PT	Air Force Undershirt
Run Time (min)	419 ± 53	429 ± 83	423 ± 63	423 ± 48
Ambient Temp (ºC)	39.1 ± 0.8	39.0 ± 0.7	39 ± 0.4	38.8 ± 0.7
Wet Bulb Temp (ºC)	71.6 ± 2.6	71.2 ± 2.7	71.5 ± 2.5	70.8 ± 2.1
Heart Rate	169 ± 18	173 ± 18	173 ± 22	173 ± 13

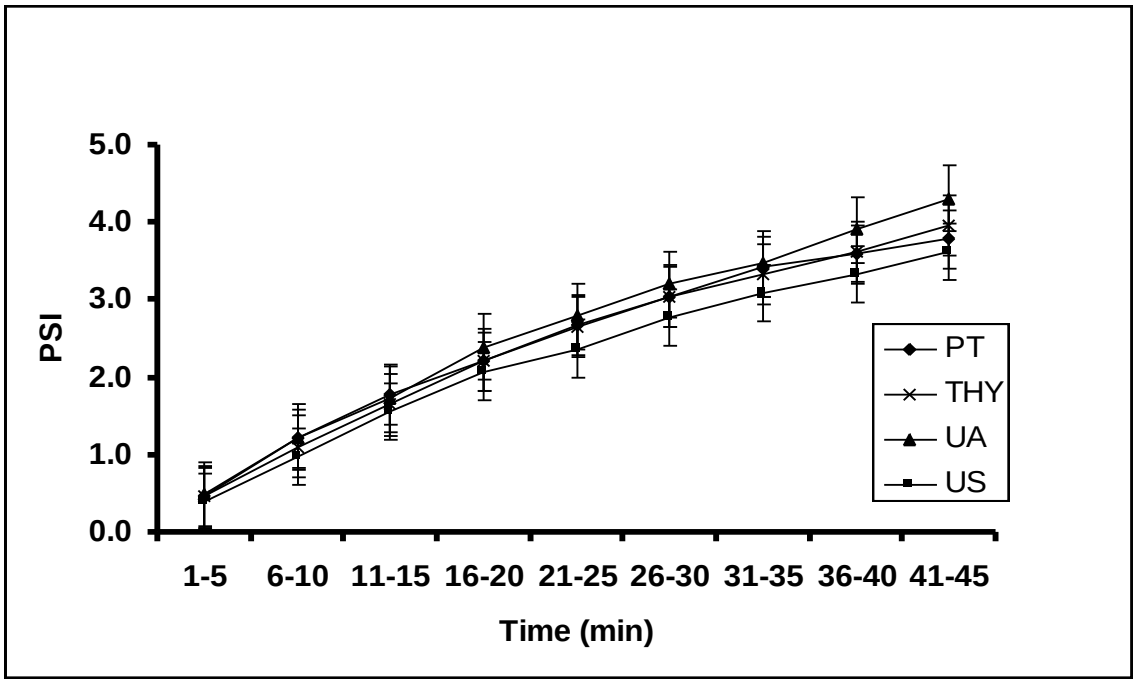

Figure 1. Five minute averages of PSI while wearing each of the four shirts.

Discussion:

For effective work/exercise in hot environments minimizing the physiological strain that is a direct result of the heat is important for both performance and safety. The working body must share total cardiac output between working muscles and the body surface. This delicate balance between providing nutrients and thermostasis provides a significant stress on the body. In many circumstances work must be sustained to complete a task or operation irregardless of environmental stress. Therefore, any assistance provided by clothing in thermal regulation would help the individual required to work in the heat. Clothing that can increase conductive, convective and evaporative cooling while reducing the impact of solar radiation could greatly assist the body in thermal regulation while working in hot environments. The four shirts tested did not show any difference in reducing the physiological stress of individuals exercising in a hot environment. The simplest explanation for the lack of difference between shirts is that all four shirts provided the same evaporative, convective and conductive cooling benefit and radiant heat protection. However, this is unlikely because shirt color was different for each shirt; black, brown, grey and white. Another explanation is that the physiological strain on the participants was high enough to negate any additional strain or potential benefit provided by the shirts. Perhaps there was a difference in the overall heat stress reduction properties of the shirts but that the body effectively compensates for the differences in the shirts. This compensation could include increased heat loss by exposed areas more adept at heat transfer, e.g. the exposed head, arms or legs.

Conclusion:

While clothing choice is an important factor to consider when preparing to exercise or work in

hot environments it seems that others factors can affect the body's ability to maintain homeostasis beyond the type of fabric covering the torso and upper arm. In conclusion the difference in heat stress between the four shirts tested was negligible indicating that no shirt tested would be more beneficial or detrimental to the individual exercising/working in a hot environment.

Field Based Studies

Field Based Study 1: First Strike Ration elicits similar blood chemistries as Meal, Ready to Eat during 3 days of field consumption.

S.J. Montain[1], J. Cuddy[2], J. Domitrovich[2], S. Harger[2], L. Harris[1], D. Slivka[2], N. Yasuda[2], B.C. Ruby[2].

[1]U.S. Army Research Institute of Environmental Medicine
Building 42, Kansas St
Natick, MA
01760

[2]Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Supplemental Funding: U.S. Army Research Institute of Environmental Medicine

Introduction:

The First Strike Ration (FSR) is a compact, light weight ration designed to be consumed during short-term high-intensity missions of approximately 3 days. It consists of 100% eat on move components that are familiar, and have high hedonic and functional acceptability scores. The purpose of this study was to evaluate if FSR sustains blood markers of metabolic and nutritional status as effectively as MRE over several days of arduous work.

Methods:

Prior to all data collection, the research protocol was approved by the institutional review board (Protocol #123-04). Eighteen active duty military performing wildland fire suppression were randomly assigned to consume either 1 FSR/day (2,864 kcal, 377 g CHO, 91 g protein) or 2 MRE per day (2,620 kcal, 358 g CHO, 84 g protein) for 3 consecutive days. Shift activity was measured by actimetry (Actical, Respironics, Inc). Food intake was assessed by diet log and collection of eaten and uneaten food wrappers. Venous blood was sampled after overnight fast (Pre) and at end of day 3 workshift (Post). Differences between diet groups were assessed using mixed model ANOVA (diet group x time).

Results:

Workshift duration was similar between diet groups (FSR: 691±36 min; MRE: 701±36 min; ±sd). Percent time performing moderate intensity work was similar over time and between groups (FSR: 30±8%; MRE: 24±10%). While percent time performing light activity was sustained in FSR (34±7%) over time, it declined on day 3 in MRE (33±8% to 25±7%). Metabolic status markers as well as nutritional status markers changed over time but were unremarkable between diet groups.

Table 1. Blood parameters pre and post 3 days of FSR and MRE consumption.

	MRE		FSR		NS = Not Significant		
	Pre	Post	Pre	Post	Diet	Time	D x T
Glucose, mg/dl	4.9 ± 0.3	4.3 ± 0.4	5.1 ± 0.6	4.3 ± 0.8	NS	p<0.05	NS
ß-HB, mM	0.02 ± 0.01	0.23 ± 0.18	0.05 ± 0.02	0.19 ± 0.33	NS	p<0.05	NS
Glycerol, mM	0.02 ± 0.01	0.04 ± 0.04	0.03 ± 0.02	0.04 ± 0.04	NS	NS	NS
FFA, mM	0.31 ± 0.24	0.62 ± 0.36	0.29 ± 0.12	0.45 ± 0.38	NS	p<0.05	NS
AST, IU/L	22 ± 8	26 ± 8	20 ± 6	22 ± 7	NS	NS	NS
Prealb, mg/dl	29 ± 5	28 ± 6	32 ± 5	31 ± 5	NS	NS	NS
BUN, mg/dl	14 ± 3	17 ± 2	14 ± 3	13 ± 3	p<0.05	NS	p<0.05
RBP, mg/dl	4.4 ± 0.8	4.2 ± 0.9	4.7 ± 0.9	4.2 ± 0.8	NS	p<0.05	p<0.05

Conclusion:

The FSR sustains metabolic and nutritional status as effectively as MRE over 3 days of arduous work.

Field Based Study 2: Proteinuria Induced by Arduous Work during Wildland Fire Suppression.

N.Yasuda[1], S.J. Montain[2], and B.C. Ruby[1]

[1]Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

[2]U.S. Army Research Institute of Environmental Medicine
Building 42, Kansas St
Natick, MA
01760

Introduction:

It has been demonstrated that proteinuria is an indicator of renal impairment that can occur following strenuous exercise (Hoover and Cromie 1981; Poortmans 1984; Riess 1979). Von Leube (1878) was the first to represent the urinary protein excretion in healthy individuals after exercise. Gardner (1956) originated the term *athletic pseudonephritis* to characterize the patterns of urinary protein excretion induced by exercise that is to be benign, transient, and reversible. Since then, proteinuria has been shown to occur after non-traumatic exercise such as rowing (Poortmans, 1990), swimming (Poortmans, 1991), marathon running (Irving et al. 1986) and triathlon (Farber et al. 1987) and traumatic sports such as football (Coye and Rosandich, 1960) and baseball (Miyai and Ogata, 1990). It has been suggested that exercise-induced proteinuria noted by the acutely abnormal urinary findings decreases and returns to the baseline within 48 hours (Gardner. 1956; Hoover and Cromie. 1981; Riess. 1979). According to Poortmans (1988), the incidence of exercise-induced proteinuria is associated with the intensity of exercise rather than the duration as noted by the relation between proteinuria and lactate.

There is the evidence that strenuous exercise may acutely induce renal disturbance but may not chronically cause glomerular and tubular disturbance (Miyai and Ogata, 1990). Urinary total protein excretion is regarded as a marker of a combination of glomerular permeability, tubular leakage, tubular secretion, and normal urinary protein excretion by the kidneys (Newman et al. 2000). Furthermore, urinary albumin (a high molecular weight protein) excretion is referred to as a reflection of glomerular permeability (Newman et al. 2000). By evaluating β_2-microglobulin (a low molecular weight protein) filtered at the glomerulus and reabsorbed by the renal proximal tubule (Miyai and Ogata, 1990; Yaguchi et al. 1998), renal tubular dysfunction can be detected in association with N-acetyl-b-D-glucosaminidase (NAG) excretion (a lysosomal enzyme) produced in proximal tubular cells (Miyai and Ogata, 1990; Yaguchi et al. 1998).

There are the growing number of full-time and seasonal wildland firefighters or individuals in the United States who are occupationally involved in arduous work duty during wildland fire suppression (Rothman et al. 1993). Previous research documents the physiological

characteristics of wildland firefighters or individuals who engaged in arduous and dangerous work associated with the difficult sleeping conditions and complex environment (Cuddy et al. 2007; Ruby et al. 2002, 2003). For instance, Ruby et al. (2002) have demonstrated the total energy expenditure of the wildland firefighters, which was characterized by relatively higher values of 3000-6260 $kcal \cdot day^{-1}$ using the doubly labeled water methodology. The arduous work duty consists of the complex working conditions such as hiking up steep terrain, digging the fire lines, brush clearing and chain sawing (Cuddy et al. 2007; Ruby et al. 2002, 2003). In such a condition, optimal strategies of fluid balance and hydration have been considered to prevent the serious medical condition such as dehydration and heat-related illness, which results in maximizing work performance (Cuddy et al. 2007; Ruby et al. 2002, 2003).

Taken collectively, the strenuous physical activity during wildland fire suppression can be categorized as among the most strenuous as demonstrated by Ruby et al. (2002, 2003). Although the reports regarding physiological responses during or after the wildland firefighting are documented, there have been no studies examining the effects of arduous occupational work on proteinuria to date. In addition, whether cumulative effects can be induced after strenuous wildland firefighting activity remains to be clarified. Due to the complex physiological changes induced by arduous work during wildland fire suppression (Ruby et al. 2002, 2003), it is plausible that there is a particular relationship between wildland firefighting and proteinuria. From the pathophysiological point of view, it is very important to provide some feedback for individuals who are engaged in wildland firefighting activity. Therefore, the purposes of this study was to examine: 1) whether poteinuria and its cumulative effects of renal impairment are observed and 2) whether a particular relationship exists between hydration markers (body weight and specific gravity) and proteinuria markers (total protein, albumin, β_2-microglobulin, N-acetyl-β-D-glucosaminidase) induced by arduous work during wildland fire suppression. It was hypothesized that work-induced proteinruia would be observed and cumulative effects would be demonstrated as arduous work is extended. Furthermore, we hypothesized that body weight loss and specific gravity would be correlated with urinary protein excretion.

Methods:

Subjects

Eighteen male active duty military served as the subjects. All participants were asked to complete a brief questionnaire and anthropometric measurement was carried out at the beginning of the work-shift. Physical characteristics of the subjects and the mean physical labor hours before this study was conducted are demonstrated in Table 1. All subjects had no history of suffering from a heat-related illness, renal, or bladder dysfunction. All participants provided written informed consent. The experimental protocol in the current study was reviewed and approved by the Institutional Review Board of the University of Montana (Protocol #123-04) and US Army Research Institute of Environmental Medicine.

Experimental Procedure

All testing consisting of three consecutive days was conducted in the Winthrop Fire Camp (Washington) in August 2006. On day 1, the ambient temperature was 10-32°C (mean 21°C) with 14-69% (mean 41%) humidity, on day 2, temperature ranged from 8-33°C (mean 21°C)

with 11-68% (mean 44%) relative humidity and on day 3, temperature was 12-36% (mean 23°C) with 8-51% (mean 33%) humidity.

All participants were allocated at random to consume one or the other of two kinds of military-based foods consisting of either one First Strike Ration (FSR) per day (2,864 kcal, 377 g Carbohydrate, 91 g protein) or two Meals Ready to Eat (MRE) per day (2,620 kcal, 358 g Carbohydrate, 84 g protein) for 3 consecutive days. Based on diet log and collection of eaten and uneaten food wrappers, food intake was assessed and then caloric intake was determined. Work-shift physical activity was determined by accelerometer (ActiCal®, Respironics, Inc.) during 3 consecutive days. Body weight and specific gravity were measured to observe each individual's hydration status over the three days of the work-shift (Helzer-Julin et al. 1988; Ruby et al. 2003). Spot urine specimens were collected at Pre and Post in both Day 1 and Day 3 in order to analyze poteinuria including total protein, albumin, β_2-microglobulin, N-acetyl-β-D-glucosaminidase (NAG, only Day 1-Pre and Day 3-Post), and creatinine. The subjects were subsequently divided into one of two groups; group A (n=9) or group B (n=9), based on the 3-day average of the percent body weight loss during the experimental period.

Urinary Collection and Body Weight Measurement

In order to reduce the variability of the excreted analyte induced by diurnal (or circadian) rhythms, the spot urine was based on the collection of the second morning urine sample after the first void urine when short sampling periods are taken into consideration (Jung 1991). Urinary sampling time ranged from 0530-0730 at pre-work-shift and 1830-2100 at post-work-shift over three consecutive work period. All urine samples were immediately frozen in the dry-ice box in order to store for the quantification of proteinuria. Frozen samples were subsequently transferred to the freezer at –80°C for the later analysis. Following complete emptying of the bladder, body weight was digitally measured.

Determination of Physical Activity using Accelerometer

To determine work activity over three consecutive days, the ActiCal® actigraphy units (MiniMitter, Bend, Oregon) were used as described by Cuddy et al. (2007). The units were securely placed on a white foam core square (~7.6 cm x 7.6 cm) to maintain them and prevent against damage for the unit. In the left chest pocket of each subject's Nomex® fire shirt, an accelerometer was secured to evaluate total body activity and movement. Due to the frequent use of upper body work duty and consistently carrying the pack, the location of chest pocket was standardized for all collection and actigraph placement to determine arduous work during wildfire suppression (Cuddy et al. 2007). Activity count data were eventually described as counts per minute (counts·min^{-1}).

Determination of Proteinuria and Specific Gravity

Total protein assay (BioAssay Systems, Hayward, CA) was conducted by an improved coomassie blue G method and subsequently determined using a photospectrometer (Milton Roy Spectronic 401, Rochester, NY). Albumin (Alpha Diagnostic International, San Antonio, TX) and the β_2-microglobulin (ALPCO Diagnostics, San Antonio, TX) were determined using the enzyme-linked immunosorbent assay, respectively. The NAG (Roche Applied Science, Indianapolis, IN) and creatinine (Stanbio Laboratory, Boerne, TX) was analyzed with the

colorimetric assay, respectively. Urinary specific gravity was used with refractometer (ATAGO, Bellevue, WA) in order to evaluate the subject's hydration status over three consecutive days.

Statistical Procedures

A two-way (group x time) Analysis of Variance (ANOVA) with mixed design (1 between, 1 within) was used to determine any significant differences in all dependent variables regarding total caloric intake, activity, and proteinuria markers. Pearson's correlation coefficient was computed between percent changes (Day 1-Pre vs. Day 3-Post) in proteinuria and hydration markers, and between urinary creatinine and specific gravity after all workshift. Furthermore, Pearson's correlation coefficient with linear regression analysis was calculated to evaluate the relationship between % body weight change (Day 1-Pre vs. Day 3-Post) and all dependent variables related to proteinuria at Day-3 Post work-shift. The overall significance was established at $p<0.05$. All data are shown as mean±SD.

Results:

Table 1. Physical characteristics of the subjects

Characteristic	Men=18
Age (yrs)	22.2±3.9
Height (cm)	179.7±7.7
Body weight (kg)	86.5±13.4
BMI (kg·m^{-2})	26.8±4.3
Physical labour hours	
previous week (hr·week^{-1})	22.4±18.0
previous 2 weeks (hr·week^{-1})	33.7±33.8

BMI=Body mass index

The group B (lower % body weight loss) showed significantly lower total caloric intake compared with group A (higher % body weight loss) – are these backwards?? Perhaps call them what they are – report the mean±sd of the % bw loss instead of describing them as lower % or higher percent (main effect for group, $p<0.05$; Figure 1-A). Furthermore, total caloric intake in both group A and B at Day 3 was significantly lower compared with Day 1 and 2 (main effect for time, $p<0.05$; Figure 1-A). In terms of activity counted by accelerometer, no significant differences were observed between either groups or time (Figure 1-B) all three days.

Urinary excretion of total protein, albumin, and NAG with non-standardization were significantly higher in group B over the experimental period (main effect for group, $p<0.05$; Figure 2-A, 2-B, and 2-D). However, no significant differences were exhibited in urinary β_2-microglobulin in either groups or time (Figure C).

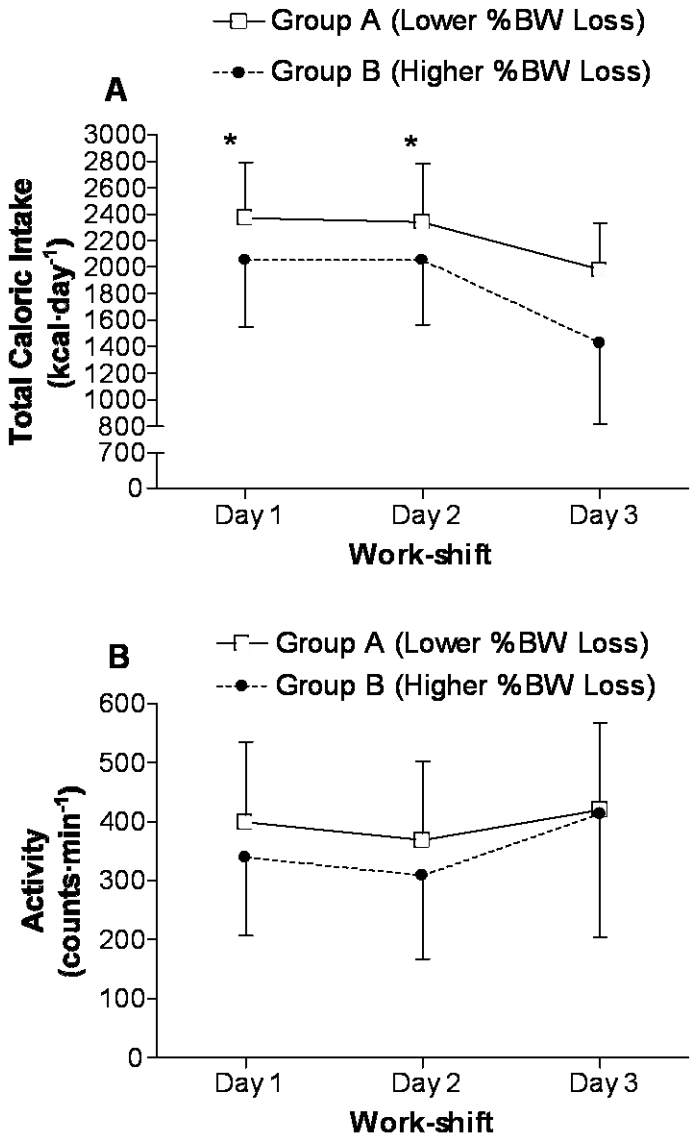

Figure 1. (A) Total caloric intake and (B) activity between lower and higher percent body weight loss groups. *Significantly different from Day 3 (main effect for time, $p<0.05$). †Significantly different from group A (main effect for group, $p<0.05$). All data are shown as mean±SD.

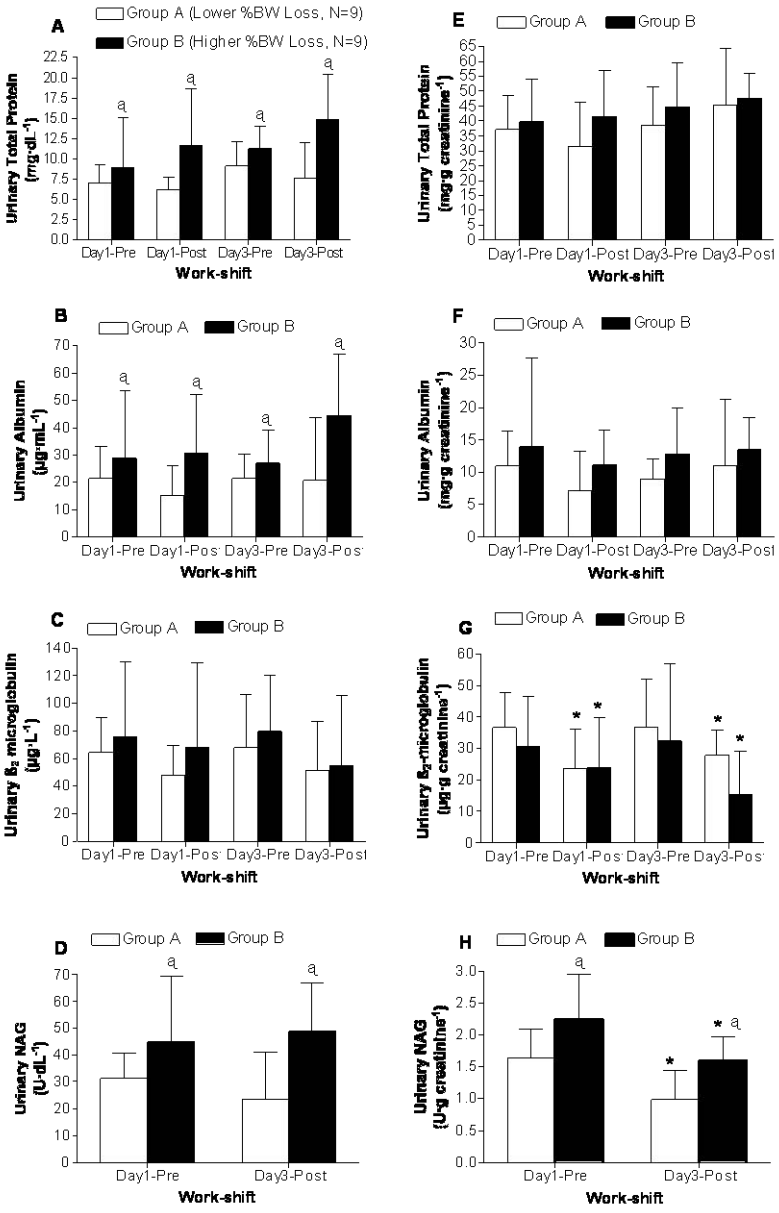

Figure 2. Non-standardized proteinuria markers: (A) Urinary total protein ($mg \cdot dL^{-1}$); (B) urinary albumin ($\mu g \cdot mL^{-1}$); (C) urinary β_2-microglubolin ($\mu g \cdot L^{-1}$); (D) urinary N-acetyl-β-D-glucosaminidase (NAG, $Ud \cdot L^{-1}$). Standardized proteinuria markers relative to urinary creatinine: (E) urinary total protein ($mg \cdot g\ creatinine^{-1}$); (F) urinary albumin ($mg \cdot g\ creatinine^{-1}$); (G) urinary β_2-microglubolin ($\mu g \cdot g\ creatinine^{-1}$); (H) urinary NAG ($U \cdot g\ creatinine^{-1}$). *Significantly different from Day 1-Pre (main effect for time, $p<0.05$). †Significantly different from Day 1-Post (main effect for time, $p<0.05$). ‡Significantly different from group A (main effect for group, $p<0.05$). All results are exhibited as mean±SD.

When expressed relative to creatinine, there were no statistical changes in urinary total protein or albumin excretion (Figure 2-E and 2-F), whereas, in the post-workshift of Day 1 and 3,

significantly lower values of urinary β_2-microglobulin were demonstrated (main effect for time between Day 1-Pre and Day 1-Post; $p<0.05$ and Day 1-Pre vs. Day 3-Post; $p<0.05$; Figure 2-G). Urinary NAG was significantly lower in Day 3-Post compared with Day 1-Pre (main effect for time, $p<0.05$), while the group B showed significantly higher excretion of urinary NAG (main effect for group, $p<0.05$; Figure 2-H).

In urinary creatinine, there were no significant differences (Figure 3-A), whereas significantly higher values of the specific gravity in group B were observed over the experimental period (main effect for group, $p<0.05$; Figure B). There was a strong correlation between urinary creatinine and specific gravity over the work-shift ($r=0.795$, $p<0.0001$; Figure 3-C).

With respect to the relation between %change in body weight (Day 1-Pre vs. Day 3-Post) and proteinuria markers at Day 3-post, urinary excretion of total protein and NAG showed significant correlations ($r=0.492$, $p<0.05$; Figure 4-A and $r=0.590$, $p<0.01$; Figure 4-D). Urinary albumin excretion tended to be correlated with %change in BW ($r=0.432$, $p=0.07$; Figure B). There was no significant correlation between urinary β_2-microglobulin and %change in body weight (Figure 4-C). When the proteinuria markers were expressed to creatinine, the relation between urinary total protein and albumin and %change in body weight were not correlated ($r=0.421$, $p=0.08$; Figure 4-E and $r=0.238$, $p=0.34$; Figure 4-F), although there is a trend for total protein excretion to be correlated with %change in body weight (Figure 4-E). The percent change in body weight was well-correlated urinary β_2-microglobulin ($r=0.596$, $p<0.01$; Figure 4-G), NAG ($r=0.552$, $p<0.05$; Figure 4-H) creatinine ($r=0.632$, $p<0.01$; Figure 5-A) and specific gravity ($r=0.556$, $p<0.05$; Figure 5-B).

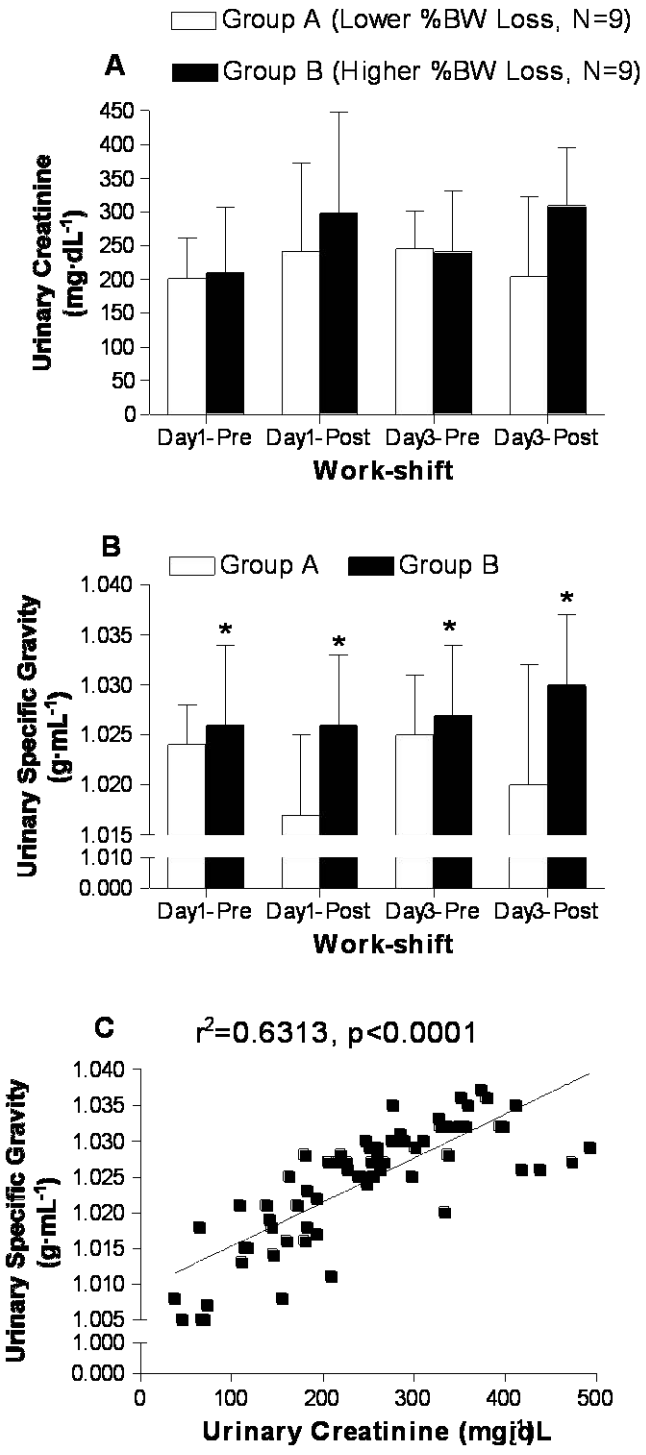

Figure 3. (A) urinary creatinine concentration ($mg \cdot dL^{-1}$); (B) urinary specific gravity ($g \cdot mL^{-1}$). *Significantly different from group A (main effect for group, $p<0.05$). (C) The relation between urinary creatinine ($mg \cdot dL^{-1}$) and urinary specific gravity ($g \cdot mL^{-1}$) ($r=0.795$, $p<0.0001$).

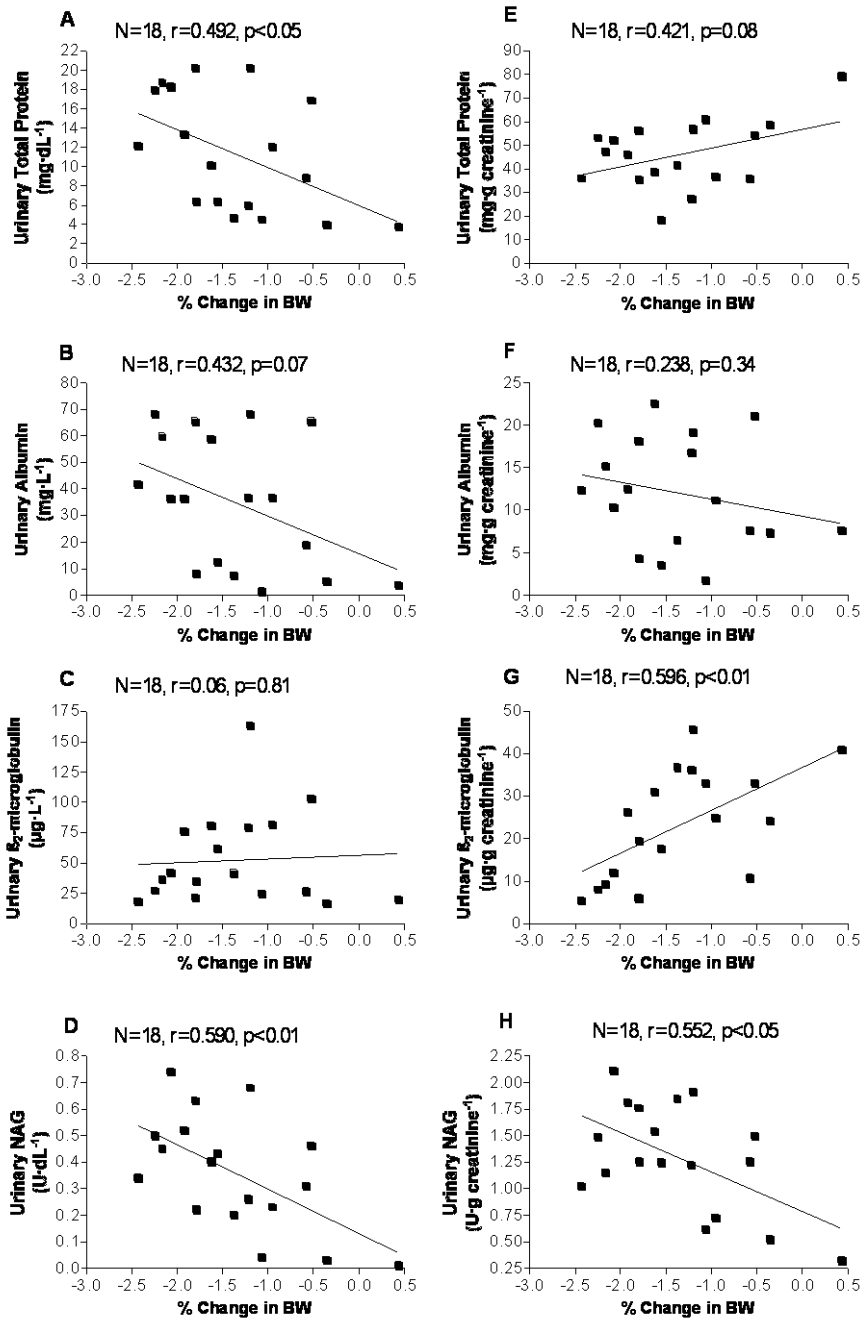

Figure 4. The correlation between percent change (Day 1-Pre vs. Day 3-Post) in body weight (body weight) and proteinruia markers at Day 3-Post: (A) Urinary total protein ($mg \cdot dL^{-1}$)(N=18, r=0.492, p<0.05); (B) urinary albumin ($\mu g \cdot mL^{-1}$)(N=18, r=0.432, p=0.07); (C) urinary β_2-microglubolin ($\mu g \cdot L^{-1}$)(N=18, r=0.06, p=0.81); (D) urinary N-acetyl-β-D-glucosaminidase (NAG, $Ud \cdot L^{-1}$)(N=18, r=0.590, p<0.01); (E) urinary total protein ($mg \cdot g$ $creatinine^{-1}$)(N=18, r=0.421, p=0.08); (F) urinary albumin ($mg \cdot g$ $creatinine^{-1}$)(N=18, r=0.238, p=0.34); (G) urinary β_2-microglubolin ($\mu g \cdot g$ $creatinine^{-1}$)(N=18, r=0.596, p<0.01); (H) urinary NAG ($U \cdot g$ $creatinine^{-1}$)(N=18, r=0.552, p<0.05).

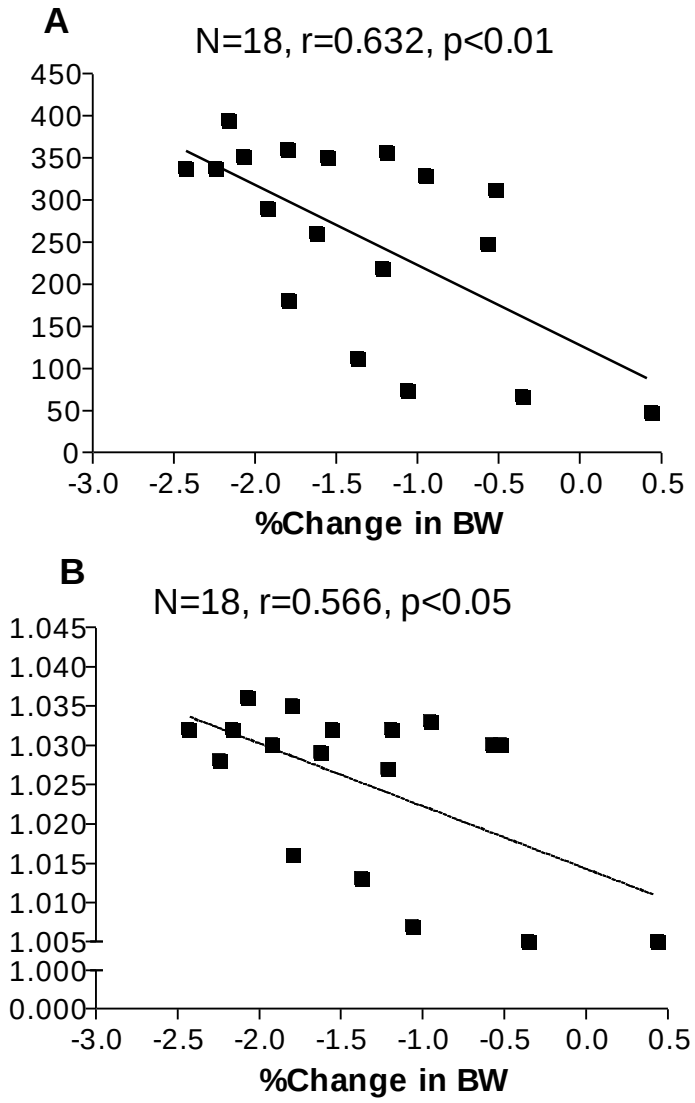

Figure 5. The correlation between percent change (Day 1-Pre vs. Day 3-Post) in body weight (body weight) and urinary creatinine and specific gravity at Day 3-Post: (A) urinary creatinine concentration ($mg \cdot dL^{-1}$)(N=18, r=0.632, p<0.01); (B) urinary specific gravity ($g \cdot mL^{-1}$)(N=18, r=0.566, p<0.05).

Table 2. Correlation between percent changes in proteinuria and hydration markers

		Total protein (mg/dL^{-1})	Albumin (µg/mL^{-1})	§$_2$-microglobulin (µg/L^{-1})	NAG (U/dL^{-1})	Creatinine (mg/dL^{-1})	Specific gravity (g/mL^{-1})	BW (kg)
Total protein	r	-	-	-	-	-	-	-
(mg/dL^{-1})	p	-	-	-	-	-	-	-
Albumin	r	0.801	-	-	-	-	-	-
(µg/mL^{-1})	p	<0.0001	-	-	-	-	-	-
§$_2$-microglobulin	r	0.423	0.506	-	-	-	-	-
(µg/L^{-1})	p	0.080	<0.05	-	-	-	-	-
NAG	r	0.604	0.654	0.565	-	-	-	-
(U/dL^{-1})	p	<0.01	<0.01	<0.05	-	-	-	-
Creatinine	r	0.749	0.789	0.855	0.682	-	-	-
(mg/dL^{-1})	p	<0.001	< 0.0001	< 0.0001	<0.01	-	-	-
Specific gravity	r	0.859	0.838	0.728	0.763	0.899	-	-
(g/mL^{-1})	p	< 0.0001	< 0.0001	<0.001	<0.001	< 0.0001	-	-
BW	r	0.520	0.524	0.426	0.553	0.696	0.569	-
(kg)	p	<0.05	<0.05	0.078	<0.05	<0.01	<0.05	-

Percent changes (Day 1-Pre vs Day 3-Post). NAG=N-acetyl-§-D-glucosaminidase, BW=body weight

Table 3. Correlation between percent changes in proteinuria

		Total protein (mg/g Cr^{-1})	Albumin (mg/g Cr^{-1})	§$_2$-microglobulin (µg/g Cr^{-1})	NAG (U/g Cr^{-1})
Total protein	r	-	-	-	-
(mg/g Cr^{-1})	p	-	-	-	-
Albumin	r	0.028	-	-	-
(mg/g Cr^{-1})	p	0.912	-	-	-
§$_2$-microglobulin	r	0.123	0.067	-	-
(µg/g Cr^{-1})	p	0.626	0.793	-	-
NAG	r	0.158	0.231	0.520	-
(U/g Cr^{-1})	p	0.532	0.357	<0.05	-

Percent changes (Day 1-Pre vs Day 3-Post). NAG=N-acetyl-§-D-glucosaminidase, Cr=Creatinine

Concerning the relation between percent changes (Day 1-Pre vs. Day 3-Post) in proteinuria and hydration markers, all urinary proteinuria and hydration markers were highly correlated (Table 2). In contrast, when proteinuria markers were expressed relative to creatinine, correlation between percent changes (Day 1-Pre vs. Day 3-Post) in proteinria markers were not significantly correlated except for the relation between β_2-microglobulin and NAG ($r=0.520$, $p<0.05$, Table 3).

Discussion:

Main Findings

The main findings in the present study were that arduous work during the wildland fire suppression induced proteinuria and tended to be accumulated as arduous work duty was extended. Furthermore, body weight loss was highly correlated with urinary protein excretion. A signicantly lower caloric intake was observed in the higher body-weight loss group compared with the lower body-weight loss group, despite similar activity counts were demosntrated in both groups.

Dehydration and Body Weight Loss

The results of this study indicated that the higher percentage of body weight loss during short-term wildland firefighting had a trend to induce relatively higher amount of urinary protein excretion associated with dehydration. This observation is explained by the fact that the intensity of exercise and the status of hydration are the factors to affect proteinuria (Carroll and Temte 2000; Helzer-Julin et al. 1988). Interestingly, the higher body-weight loss group in the current study showed a significantly lower caloric intake compared with the lower body-weight loss group, which is supported by the previous evidence that body weight loss resulted from an overall energy deficit and decreased total body water (Mudambo et al. 1997; Ruby et al. 2003). However, estimated work activity counted by accelerometer were similar in both higher and lower body weight loss groups over three consecutive days in this study. Previous data have demonstrated the energy demand of wildfire suppression (Ruby et al. 2002). In the study, some subjects could maintain energy balance, while others lost body weight up to 1.7 kg over the five-day work period (Ruby et al. 2002). In line with those findings, the mean specific gravities in all participants tended toward being dehydrated (>1.020 $g \cdot mL^{-1}$) over the period of the study. Furthermore, some individuals' body weight loss decreased up to 2.4% after the 3-day work-shift in the present investigation. Body weight measurement has been considered to be a simple and advantageous marker in order to determine fluid balance (Cheuvront et al. 2002, 2004). In this regard, a cut-off values for euhydrated condition based on body weight measurement has been shown as <1% change (American College of Sports Medicine, 2007). In addition, it has been suggested that dehydration more than 2% of body weight change can attenuate aerobic work performance and induce mental/cognitive performance (Cheuvront et al. 2003). Thus, it is very important to maintain dehydration to optimize work performance (Cuddy et al. 2007; Ruby et al. 2002, 2003). However, as wildland firefighting is subjected to unpreditable and extreme condisions consisting of strenuous muscular work associated with psychological and physiological stress (Ruby et al. 2002, 2003), it may have been difficult to ideally maintain euhydrated body conditions (≤ 1.020 $g \cdot mL^{-1}$, Alessio et al. 1985; Armstrong et al. 1994; Popowski et al. 2001).

Glomerular and Tubular Function in the Kidney

With regard to proteinuria, previous research has demonstrated that the intensity of exercise is in concord with increased levels of urinary protein excretion, as characterized by the strong correlation between proteinuria and lactate (Poortmans et al. 1988). Therefore, our results indicate that the physical demands of wildland firefighting appears to be sufficient to induce proteinuria, which is supported by the fact that strenuous physical activity augments urinary protein excretion level (Poortmans 1985). In the current investigation, the elevation in urinary protein excretion may have been attributed to arduous work-related stress including body weight loss and dehydration (e.g. sweating, Nadel 1979). Although normal urinary protein excretion has been suggested as <10 ml·dL^{-1} (Carroll and Temte 2000; Helzer-Julin et al. 1988), there was a tendency for dehydration to induce more urinary excretion of protein than normal values in this study. The higher proteinuria excretion is likely to be characteristic of individuals who are engaged in wildland firefighting consisting of the complex upper- and lower-body involvement.

The excretion of urinary proteins including total protein and albumin increases following strenuous exercise (Poortmans 1985). In addition to those high-molecular weight proteins, low-molecular weight proteins such as β_2-microglobulin are also considered to be elevated after the heavy physical work (Poortmans 1985). Given a higher excretion of urinary total protein, albumin, and β_2-microglobulin, it is possible that post-exercise proteinuria reflects mixed glomerular and tubular impairment (Ohno et al. 1993; Poortmans 1985). According to Poortmans (1985), glomerular permeability, tubular reabsorption and disposal of the absorbed proteins can be three variables involved in urinary protein excretion. In this respect, the post-exercise proteinuria has been considered to result from the increased glomerular permeability and/or impaired tubular reabsorption (Poortmans 1985). For instance, the elevation of glomerular permeability is explained by a result of the appearance of high-molecular weight proteins in the urine (Yaguchi et al. 1988; Poortmans 1985). Moreover, the impairment of tubular function is demonstrated by the recognition of the low-molecular weight proteins in the urine (Yaguchi et al. 1988; Poortmans 1985). The NAG (a lysosomal enzyme) produced in proximal tubular cells has commonly been used with β_2-microglobulin in order to examine renal tubular dysfunction, as explained by the observations that the filtered amounts of β_2-microglobulin with NAG surpass the ability of reabsorption in the tubules (Miyai and Ogata, 1990; Poortmans 1985; Yaguchi et al. 1988).

In the current investigation, percent body weight loss (Day 1-Pre vs. Day 3-Post) in all subjects was correlated with urinary excretion of total protein, albumin and NAG at Day 3-Post. In line with those results, there was a trend for %change in hydration markers (Day 1-Pre vs. Day 3-Post) to be well-correlated with %change in all proteinuria markers (Day 1-Pre vs. Day 3-Post). When proteinuria markers are relative to creatinine, a significant correlation was found between post-exercise NAG to creatinine ratio and β_2-microglobulin to creatinine ratio (Day 1-Pre vs. Day 3-Post), which may be due to reflection of the extent of tubular disturbance. Although our results were in agreement with the previous finding in short-course triathlon (Yaguchi et al. 1998), some research reports no correlation between β_2-microglobulin and NAG after baseball training camp (Miyai and Ogata, 1990). In addition to those observations, there was no significant correlation between post-exercise urinary total protein to creatinine and NAG to

creatinine ratio (Day 1-Pre vs. Day 3-Post) in the present study. Furthermore, similar results were also observed for urinary total protein to creatinine and β_2-microglobulin to creatinine ratio (Day 1-Pre vs. Day 3-Post). Those phenomena are consistent with the previous findings (Yaguchi et al. 1998), whereas other studies shows positive correlation without the creatinine-adjustment (Miyai and Ogata). The urinary excretion of enzymes or proteins expressed relative to urinary creatinine can at least in part be adjusted to reduce the variability such as inconstant dilution and concentration of urine samples (Elkins et al. 1974; Jung et al. 1986). However, such a creatinine-based adjustment may bring about another disadvantage. For example, as the ratio of protein to creatinine comes from two variables, such a ratio appears to have an influence on urinary creatinine excretion dependent on sex, age, muscle mass, and urine flow rate (Jung 1991).

Taken together, our findings indicate that the degree of glomerular dysfunction may not always be associated with the tubular impairment as reported previously (Yaguchi et al. 1998). In this regard, further research is required to clarify the mechanisms between glomerular and tubular disturbance induced by repeated and long-lasting strenuous exercise. In parallel with the aforementioned observations, the plausible reasons for the observations in the current study may be related to the presence of several factors, which are renal ischemia and vasoconstriction (Castenfors 1977; Javitt and Miller 1952; Poortmans 1985), increased renal vein pressure (Bruce 1972; Riess 1979), and bladder contusions (Blacklock 1977; Fred 1978; Fred and Natelson 1977).

In conclusion, our findings in the present study indicate that wildland firefighting appear to cause and accumulate proteinuria as the arduous work prolongs. Moreover, the higher percent of body weight loss accompanied by dehydration after wildland firefighting may increase renal protein excretion. Although arduous work appears to acutely induce proteinuria as a result of a daily work output, whether long-lasting exposure to strenuous wildland firefighting activity induces a chronic glomerular and tubular damage still remains inconclusive. Therefore, further research is warranted in this regard. It is suggested that strenuous work duty should be taken into account with caution in order to protect against chronic renal dysfunction.

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Field Based Study 3: Muscle Glycogen Utilization During Wildfire Suppression

T.J. Tucker, J.S. Cuddy, D.R. Slivka, W.S. Hailes, and B.C. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

The energy demands associated with physically demanding occupational tasks, such as military training[9], mountaineering[10, 11], and wildland fire suppression[3, 4, 6, 7], have been well documented. It has been previously determined that the energy expenditure associated with wildland fire suppression ranges from 12-26 $MJ \cdot day^{-1}$ (2868 – 6214 $kCal \cdot day^{-1}$)[7]. Additionally, the daily water turnover rates (over 5 days) in Wildland Firefighters (WLFF) averaged 6.7 ± 1.4 $L \cdot d^{-1}$ (94.8 ± 24.1 $ml \cdot kg^{-1} \cdot d^{-1}$)[6]. While these numbers indicate daily work efforts higher than most job occupations, the majority of time spent during wildland fire suppression is sedentary, accounting for approximately 61-66% of work time[3]. Most of the work activity is at a light intensity, with intermittent bouts of moderate and/or vigorous activity.

Dietary habits during wildland fire suppression can vary based upon the location of the fire and the urgency of the suppression efforts. Firefighters can stay at a base camp where catered food is available for breakfast and dinner, and sack lunches are provided for lunch. Wildland firefighters can also be located outside of an organized fire camp (spike camp), meaning they set up a self-sufficient camp in a remote location and eat meals-ready-to-eat (MRE) for breakfast, lunch, and dinner unless other meals are transported in. *Ad libitum* dietary patterns for WLFF typically include a higher intake of dietary protein and fat than recommended for arduous work[7]. Our laboratory has previously determined that supplemental provisions of foods and drinks rich in carbohydrates are effective at improving and/or maintaining a more consistent work output during shifts[3].

The specific job tasks of wildland firefighters vary from day to day and from hour to hour, often in hostile environmental conditions and steep, mountainous terrain. On certain days an entire crew will construct fire line (removal of topsoil to construct a fire break to prevent further spreading) from the start of a work shift until the end of the work shift. However, on a typical work day a 20 person wildland fire crew will divide into several smaller squads composed of 4-5 people. These squads have separate tasks, such as sawing and clearing debris, digging line, scouting, and laying hose. Within each squad certain members have different tasks, such as lookout, further varying the day to day job demands. Thus, there are not uniform physiological demands among the crew members each day.

A low carbohydrate diet over consecutive days while engaging in exercise training can lead to reduced resting muscle glycogen levels[2]. For WLFF, the combination of insufficient CHO intake and varied work demands over the course of a fourteen day rotation provides the framework for fluctuations in intramuscular fuel sources, especially muscle glycogen.

Beginning work tasks or exercise with inadequate muscle glycogen stores has been shown to decrease exercise time to exhaustion, while increasing the perception of effort. It is critical for optimal safety that WLFF have adequate nutritional intake to make appropriate decisions and avoid dangerous situations, as well as have the energy available to escape hazardous circumstances should they arise.

The purpose of this project was to determine the effects of wildfire suppression on muscle glycogen utilization in male and female wildland firefighters. The information collected in this study will help describe patterns of glycogen use during extended work shifts. This knowledge will help the wildland firefighter or others who participate in extended work shifts make appropriate nutritional decisions for optimal performance, recovery, safety, and health.

Methods:

Subjects

Subjects included both male (n=10) and female (n=2) Wildland Firefighters (86 ± 9 kg, 183 ± 8 cm) from a Type I Hotshot crew in Western Montana. Subjects were recruited prior to the 2008 fire season at an informative meeting. Prior to participation all subjects read and signed the consent form previously reviewed and approved by the University of Montana Institutional Review Board (IRB) (Protocol #63-07). Each participant wore and carried traditional WLFF gear including: Nomex long-sleeved shirt and pants, mid-calf leather logging boots, a 100% cotton short-sleeve undershirt, leather gloves, hard hat, and a 12 – 20 kg pack. Daily firefighting tasks included hiking over varied terrain, constructing fire-line, and managing hose.

Research Design

Upon arrival to a fire in Northwestern Montana, subjects were randomly assigned into two groups (n=6). Subjects arrived to the mobile laboratory at 0600 n a fasted state. Subjects were weighed wearing Nomex pants, leather boots, and an undershirt (~5 kgs). After descriptive data were collected, a muscle biopsy was collected from the vastus lateralis. Subjects were then equipped with an activity monitor (ActiCal®) and were provided with instructions regarding accurate recording of dietary intake. After the work shift, body weight was recorded with the subject wearing the same clothes as at 0600 and a post-shift muscle biopsy was taken. In addition to collecting food logs, each subject was interviewed regarding the food records in order to ensure accuracy.

Weight

Weights were taken on a calibrated *Ohaus CW-11*, Pinebrook, NJ.

Muscle Biopsies

Biopsies were taken pre- and post- work shift from the *vastus lateralis* muscle of the same leg using a 4-5 mm Bergstrom percutaneous muscle biopsy needle [1]. The second biopsy was obtained from a separate incision 1.5-2 cm proximal to the previous biopsy. After any excess blood, connective tissue, or fat were removed, tissue samples were immersed in liquid nitrogen and stored at -80°C for later analysis.

Actigraphy

Activity counts were obtained using ActiCal® activity monitors (MiniMitter, Bend, OR). The monitors were initialized and distributed to crew members to determine activity counts during one day of firefighting. Researchers placed ActiCals® in the left chest pocket of the Nomex fire shirt. For protection, stability, and in order to keep the unit in a secure position, each monitor was secured in a white foam core square (~7.6 cm x 7.6 cm). This location was chosen due to the amount of upper body movement associated with WLFF. In addition, researchers equipped subjects' bootlaces with a second monitor to measure the amount of lower extremity movement. Activity counts were averaged into one-hour intervals and were expressed in counts·min^{-1}.

Diet

Participants were allowed to eat and drink *ad libitum* throughout the day. Each subject was provided a notebook and pencil to record foods consumed. For analysis, nutrition fact labels were used when available. In addition, The Food Processor Nutrition and Fitness Software (ESHA Research, Salem, OR) program was used when food items did not have nutrition fact labels.

Muscle glycogen analysis

Muscle glycogen was analyzed using an enzymatic spectophotometric method. Samples were weighed (15.7 ± 0.6 mg wet weight) upon removal from an -80°C freezer and placed in 0.5 ml, 2N HCl solution. The sample solutions were weighed, incubated for two hours at 100°C in an oven, then re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5ml of 0.67 NaOH was added. A volume of this muscle extract (20µl) was added to 1 ml of Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read on a spectrophotometer at 340nm. Muscle glycogen was then calculated using the extinction co-efficient of NADH. Muscle glycogen concentrations are expressed in mmol · kg^{-1} wet weight of muscle tissue. The coefficient of variation for duplicate samples was 2.4 ± 0.4% (mean ± SE).

Results:

Body Weight

There was a significant decrease in body weight on Day 1, 89.6 ± 8.4 to 89.0 ± 8.3 pre and post, respectively, $p<0.05$. Body weights were similar pre to post shift on Day 2, 83.3 ± 9.0 to 83.5 ± 8.9 pre and post work shift, respectively.

Muscle glycogen

Muscle glycogen decreased significantly from pre to post work shift on Day 1 (group 1), 115 ± 14 to 82 ± 23 mmol·kg^{-1} wet wt., $p<0.05$, Fig. 1. Muscle glycogen was similar from pre to post work shift on Day 2 (group 2), 83 ± 16 and 82 ± 14 mmol·kg^{-1} wet wt., Fig. 1. Muscle glycogen was higher Day 1 Pre compared to Day 2 Pre, 115 ± 14 versus 83 ± 16 mmol·kg^{-1} wet wt., respectively, $p<0.05$, Fig. 1.

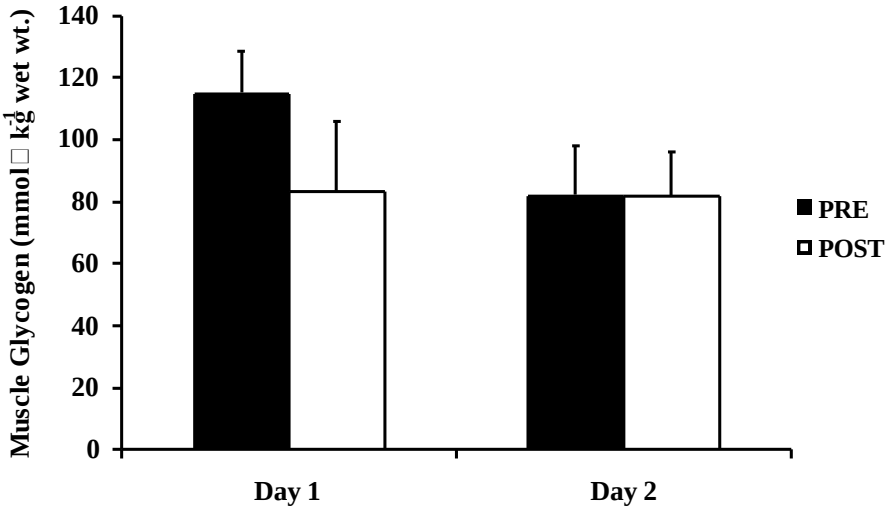

Figure 1. Muscle glycogen utilization during the work shift. *different than Day 1 pre, $p<0.05$.

Activity patterns

Activity patterns were similar on Day 1 and Day 2, 191 ± 40 compared to 241 ± 134 counts·min^{-1}, respectively, Fig. 2. There were no differences between days for percentage of time spent at sedentary (72% ± 8), light (25% ± 6), and moderate/vigorous (3% ± 3) intensities.

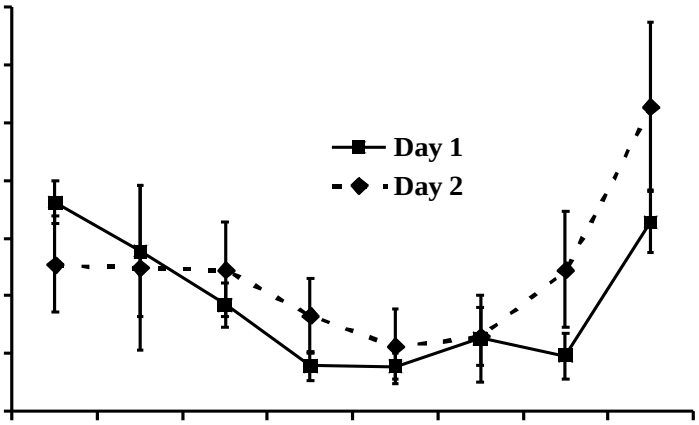

Figure 2. Activity patterns during the work shift for Day 1 and Day 2.

Dietary intake
Total kilocalorie intake was greater on Day 1 compared to Day 2, 2562 ± 623 versus 1754 ± 484 kcals·d^{-1}, respectively, $p<0.05$. Total carbohydrate intake was higher on Day 1 compared to Day 2, 367 ± 83 versus 258 ± 43 grams·d^{-1}, respectively, $p<0.05$. Carbohydrate intake was similar between Days 1 and 2, amounting to 4.1 ± 1.0 and 3.1 ± 0.5 g·kg^{-1}·day^{-1} for Days 1 and 2, respectively.

Discussion:

This project is unique in that it is the first study to collect muscle biopsies from wildland firefighters during actual wildfire suppression when participants self-select work output and consumed food *ad-libitum*. The primary finding of the current study is the significantly higher pre work shift muscle glycogen stores for subjects on Day 1 (115 ± 14 mmol·kg^{-1} wet wt.) compared to subjects on Day 2 (83 ± 16 mmol·kg^{-1} wet wt.). Additionally, the diversity in muscle glycogen change (-64 to +20 mmol·kg^{-1} wet wt.) from pre to post shift demonstrates the variability of job tasks and self-selected food intake patterns during the work shift and during the post-shift recovery period. These findings demonstrate the diversity of activity patterns, self-selected nutritional intake, and the potential to inadequately recover and maintain normal levels of muscle glycogen from day to day. This has direct relevance to the military since subjects' meals were nearly exclusive MRE.

Daily activity counts ranged from 78 to 456 counts·min^{-1}, with a mean of 198 ± 99 counts·min^{-1}, indicating the diversity of a WLFF's work tasks. Diversity in activity is not only determined by the size and age of the fire, terrain that a fire is in, but also by the job designated to the individual WLFF. Even when fire activity and suppression efforts are the highest for most WLFFs, some crewmembers are assigned to lookout duty and are less active. It was observed that the self-selected work habits of the WLFF are very diverse, indicated by the wide range of activity counts and the large standard deviation. There was no difference in activity counts between subjects on Day 1 and Day 2, indicating similar levels of work between the two work shifts. The majority of time was spent in the sedentary category (72%), while 25% was spent completing light activity. Only 3% of the work shift included moderate/vigorous activity. These findings are in agreement to previous research done with similar wildland fire suppression crews[3]. Despite the amount of time spent in the sedentary category and *ad-libitum* feeding, muscle glycogen demonstrated a decrease throughout the work shift.

Food items consumed consisted of a variety of MRE's and sack lunches provided by a catering service. Briefly, sack lunches contain food items provided by the caterer, such as 1-2 sandwiches, juice, fruit, potato chips, cookies, etc. The nutritional data collected represents the food consumed between biopsies (breakfast and shift food). Participants from Day 1 ate a significantly greater amount of kilocalories (30%) and carbohydrates (32%) compared to participants on Day 2, yet had a greater mean decline in muscle glycogen (33 mmol·kg^{-1} wet wt. versus 1 mmol·kg^{-1} wet wt. for Days 1 and 2, respectively) despite similar activity levels. A probable explanation for this difference in mean change during the day was the participants on Day 2 arrived with reduced muscle glycogen compared to participants on Day 1. Glycogen losses incurred on the first day were probably not sufficiently replenished during the evening

prior to Day 2.

Typically, fire crews return to base camp after a work-shift and consume a fully catered, all-you-can-eat dinner. Additionally, they consume an all-you-can-eat breakfast in the morning. The crew in the current study was located in a remote location with limited resources and did not return to base camp each evening to eat dinner or breakfast the following morning. Food availability when "spiked out" is often limited to MRE's or supplemental provisions provided by the crew. One possible explanation is that consumption of MRE's may inadequately re-supply muscle glycogen and create negative energy balance over time. The only source of kilocalories in the evening was those provided by MRE's. Thus, the participants may have consumed fewer kilocalories as a result of the monotony of food items and palatability of the meals. Or, the MRE's provide a disproportionate percentage of substrates required for extended work (36% FAT, 53% CHO, and 12% PRO). Previous research has demonstrated that for endurance athletes, CHO intake should represent 60-70% of total kilocalories. For the typical MRE, the amount of carbohydrate is 7-17% less than this recommendation.

Previous research has quantified total daily energy expenditure of wildland fire suppression to be 66 and 55 kcals·kg^{-1} for males and females, respectively[7]. In the current study, total energy intake during the work shift was 26 and 20 kcals·kg^{-1} for males and females, respectively (61% and 64% below prior research). This means that to achieve the kilocalorie needs as previously reported, each WLFF would have to consume two complete MRE's in the evening to replenish depleted fuel sources, primarily muscle glycogen. If subjects do not replenish depleted energy stores, as indicated by the low pre-glycogen levels on Day 2, their ability to maintain work effort may be compromised, which may also influence overall safety on the fireline. A strategy to maintain or increase muscle glycogen during prolonged activity is to feed more aggressively during the workday. In previous research from our laboratory, subjects consumed placebo or CHO during 10 hours of upper body, cycle and treadmill exercise[5]. When fed CHO, muscle glycogen loss was minimized by 52% compared to placebo feeding. Another strategy to replenish fuel sources would be to focus on a high CHO diet post shift. However, when only MRE's are provided this is a formidable task due to a low level of CHO (53%), a poor variety and flavor of CHO foods (i.e. bread, crackers, etc).

Thus, higher quality, quantity, and diversity of foods need to be provided to WLFFs at remote locations (spike camps) to mitigate the deleterious effects of low muscle fuel stores in an effort to maximize performance and maintain safety on the fireline. By having insufficient CHO consumption, WLFFs may have difficulty maintaining the standard fire orders: "Be alert. Keep calm. Think clearly. Act decisively" and "Fight fire aggressively, having provided for safety first." [8]

A considerable limitation of this study is not knowing dietary intake or activity patterns for subjects the evening prior to their morning muscle biopsy. Nonetheless, by participants arriving on Day 2 with a mean of 83 mmol·kg^{-1} wet wt., it indicates an inadequate feeding strategy during the day and/or evening prior. Another limitation of this study is the inability of the ActiCal to accurately record work output when the subject is climbing steep terrain. The current fire required that the WLFF work in this terrain, which increased the difficulty of the job. The ActiCal will read low activity counts because the participant is moving slowly, even though they

are hiking steep terrain and carrying equipment. Thus, it makes it difficult to compare activity data from this study with previous wildland fire research that uses the ActiCal as a measure of work output.
The purpose of the current study was to determine the effects of wildland firefighting on muscle glycogen utilization. Wildland firefighting is an inimitable situation in which multiple factors, such as work output and dietary intake, not only vary from person-to-person, but also from day-to-day.

This study demonstrates the variety of self-selected nutritional and activity habits of WLFFs, and questions the adequacy of the current policies on the provisions of MRE's and other supplemental foods during sustained fire suppression operations in remote locations. Wildland firefighters need to feed aggressively throughout the work day and in the evenings with high carbohydrate food sources in order to replenish depleted fuel sources. This is especially important if crews are not located in fire camps and are forced to rely on limited provisions.

Implications of this study extend to other occupations that require physical effort and sound decision-making, such as the military. It is imperative for soldiers, airmen, and marines to be well nourished during and following training and active duty combat in order to maintain muscle glycogen levels to insure their safety.

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Field Based Study 4: Effects of Beta Glucan on Symptoms of Upper Tract Infection in Wildland Firefighters.

J.W. Domitrovich, S.G. Harger, and B.C. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Supplemental Funding: Biothera

Introduction:

Wildland firefighters (WLFF) experience long and arduous work conditions (23). Work shifts can last 16 hours a day or longer, depending on the fire, repeated for 14–21 days. Increased work output, stress, high ambient heat, compromised dietary intake, particulate matter inhalation, and sleep deprivation experienced by WLFF increase the risk of upper respiratory tract infection (URTI) development. Our lab has demonstrated that WLFF expend 4,000–6,000 kcal·day^{-1} and turn over 6 liters of water per day (22;23), similar to the energy expenditure of marines during combat training. Increased work loads, and decreased immune function increase susceptibility to URTI (25), for review see (8).

During the wildfire season, WLFF hand crews live, eat and sleep as a unit in close quarters. The symptoms of URTI include sore throat, runny noise, coughing, sneezing, fever, headache, and colored sputum and mucus. Once a WLFF begins to feel symptoms of URTI they may try to prevent the infection and continue working by taking large doses of vitamin C. A recent study in ultra-marathon runners suggests that 600 mg of vitamin C taken 21 days before and after a 90 km race reduces symptoms of URTI (20).

Unpublished findings from our laboratory found a decrease in salivary IgA levels when WLFF were not provided with adequate carbohydrate during the work shift. Previous research suggests that carbohydrate supplementation can moderate salivary IgA concentration during prolonged exercise (4). A reduction in salivary IgA may contribute to URTI risk (15). Once an individual develops a URTI, it can easily spread to other members of the crew. If a crew's roster falls below 18 individuals they can no longer operate as a hand crew, which can lead to financial losses to crewmembers and a loss of productivity to overhead management personnel.

A novel approach to infection control is the use of biological response modifiers that enhance the innate immune response without expressing damaging pro-inflammatory cytokines (13;16). Beta glucans are glucose polymers derived from a variety of sources including yeast, grain, or fungus. In general, beta glucans act as biological response modifiers to enhance the immune system responses to infectious organisms. Many studies report that beta glucan significantly increases *in vitro* microbicidal activity of human neutrophils and macrophages against a variety of pathogens without directly stimulating synthesis of cytokines such as IL-1 or TNF-α (3;10;12). *In vivo*, beta glucan reduces sepsis by enhancing leukocyte number, and bacterial phagocytosis and

killing (16;19). *In vivo* beta glucan, acting in synergy with the antibiotic cefazolin, prevented staphylococcal wound infection in guinea pigs and increased neturophil number compared to saline treated controls (11). In clinical trials, beta glucan reduces postoperative infection rates and shortens intensive care unit stay duration (1;2;7).

Beta glucan could be a novel mechanism to help incident management teams maintain crew health during wildfire suppression. The purpose of the current investigation was to determine the effects of a beta glucan immunomodulating supplement on symptoms of URTI in WLFF during 14 days of work.

Methods:

Subjects

Subjects were recruited by telephone calls to WLFF hand crews in Forest Service geographical regions I, III, V and VI (Northern Rockies, Eastern Basin, California and Northwest), prior to 2007. When possible, researchers met with crews face-to-face and outlined the study; otherwise it was completed via a phone call. Subjects' average age was 26 ± 4 years, height 178 ± 8 cm, and weight 80 ± 12 kg. Subjects completed a Physical Activity Readiness Questionnaire (PAR-Q) prior to data collection, and provided written informed consent by completing a University of Montana Institutional Review Board (IRB) approved consent form. Research was accomplished under Protocol 58-07.

Fifty-six out of 97 subjects returned completed questionnaires, for a completion rate of 58%. Reasons for withdrawal from the study included poison ivy (n = 4), inhalation of retardant dropped from air operations (n = 3), nausea during active phase (n = 2), crew resignation, lack of access to supplement due to assignment (n = 14), and one crew had a crew carrier break down and lost their completed forms (n = 19).

Research Design
Subjects were blinded to condition. Each treatment period used a randomized crossover design, with subjects acting as their own controls. Supplements consisted of 500 mg beta glucan (Biothera, The Immune Company, Eagan, MN), or a similar placebo capsule that was consumed daily. Subjects completed two 14-day conditions with a 3-day washout between treatments. During the study, subjects were not allowed to ingest any immune boosting supplements.

Data Collection
Subjects completed a previously validated daily health log adapted from Nieman et al. (17) during each treatment. In addition to the questions used by Nieman et al. (18), additional questions were added specifically pertaining to the supplement. An individual was classified as having a URTI when they recorded 2 (cold) or 3 (flu) symptoms for 2 or more consecutive days (17). Other illnesses or symptoms were classified in the same manner. At the conclusion of each treatment period subjects completed an overall health performance questionnaire, this contained questions regarding subjects' perceived overall health during the 14-day treatment.

A subset of 24 subjects wore accelerometers (Actical©, MiniMitter, A Respironics Company,

Bend, OR) in their left shirt pocket as described by Cuddy et al. (5) to monitor work output. This was used to determine whether the work output during the work shift, and over a 24-h period, was different between conditions. Determination of work shift was based on crew time on the clock during that calendar day. Activity was averaged during each work shift and classified into 4 intensity levels: sedentary (0–99 counts/minute), light (100–1499 counts/minute), moderate (1500–6499 counts per minute), and vigorous (more than 6500 counts/minute) (9).

Data Analysis

A Wilcoxon's signed rank test was used to evaluate symptom differences in the daily health questionnaires. A student's t-test was used to determine differences in illness perception during each treatment. A 2-way repeated measures analysis of variance (ANOVA) was used to analyze activity data over the 14-day treatment. Statistical analysis was conducted using SPSS software (SPSS, INC. Chicago, IL) and significance was set at $p < 0.05$.

Results:

All descriptive data are expressed as mean ± SD. There was a statistical trend towards a lower incidence of URTI symptoms per subject from the daily health logs during the beta glucan phase compared to the placebo, with 19 (37%) and 26 (48%) subjects reporting URTI symptoms, respectively ($p = 0.07$). There were no differences in reported symptoms of other illnesses or conditions from the daily health log (Table 1). Other symptoms reported during the 14-day trials are reported in Table 2.

Table 1. Daily health symptom incidence not related to URTI

Symptom	Beta Glucan Total (%)	Placebo Total (%)	Wilcoxon Signed Rank Test
Nausea or Vomiting	3 (6)	3 (6)	0.317
Muscle or Bone Problems	6 (11)	8 (15)	0.480
Allergy Symptoms	8 (15)	9(17)	0.102
Sinus Infection	6 (11)	4 (8)	0.655
Ear Infection	1 (2)	0 (0)	0.317
Skin Infection	1 (2)	1 (2)	1.00
Other Health Problems	2 (4)	3 (6)	1.00

During the course of the study, subjects reported a higher perception of health during the beta glucan condition (5.36 ± 0.23) than during the placebo phase (4.65 ± 0.22; $p = 0.01$). There were no differences in the number of instances when subjects could not perform normal physical activity ($p > 0.05$). When the subjects were asked to rate their overall health over the supplement regimen compared to the start of the trial, WLFF could not distinguish between the beta glucan phase (5.4 ± 1.9) and the placebo phase (4.8 ± 1.7) and reported no difference in feelings of overall health ($p = 0.07$).

Table 2. Reported illnesses or conditions from health assessment questionnaire

Condition	Beta Glucan Total (%)	Placebo Total (%)
Visual Changes	0 (0)	3 (6)
Headache	31 (51)	20 (40)
Dizziness	4 (8)	2 (4)
Thirst	13 (25)	11 (22)
Fatigue	24 (45)	19 (38)
Skin Problems	7 (13)	8 (16)
Fever	2 (4)	6 (12)
Sinus Irritation	28 (53)	19 (38)
Ear Infection	8 (15)	4 (8)
Increased Allergies	12 (23)	6 (12)
Other	4 (8)	5 (10)

Work shift activity is presented in Figure 1. There was no time by treatment interaction (beta glucan= 155 ± 133 counts/min, placebo = 180 ± 130 counts/min for activity) ($p>0.05$). However, there was a main effect for time during days 3–14 compared to day 1 ($p < 0.05$), indicating an increased activity in days 3–14 *versus* day 1. There were no differences between groups for activity counts over a 24 hour period ($p > 0.05$).

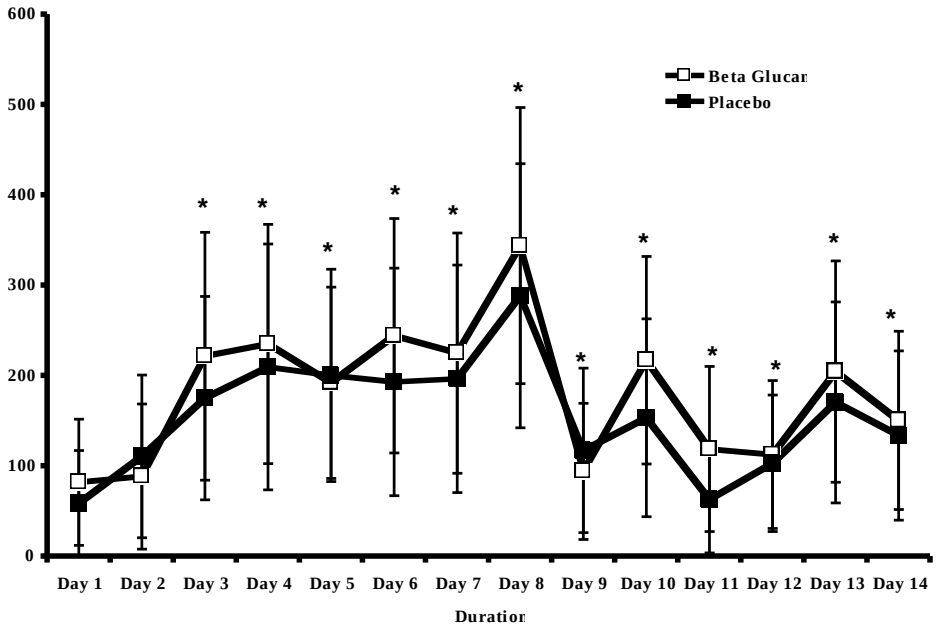

Figure 1. Average activity counts per work shift during the 14-Day trials, mean ± SD. *=Main effect for time for days 3-14 versus day 1 ($p<0.05$).

Discussion:

This is the first report of the possible effects of a beta glucan supplement on URTI symptoms in WLFF. The current study shows a non-significant statistical trend for decreased symptoms of URTI in WLFF during 14 days of beta glucan administration. There were no differences in other possible benefits, such as improved allergy symptoms or fewer sinus infections, during the beta glucan administration (Table 1).

Beta glucan improves immune function in a variety of animal models, and does not increase pro-inflammatory cytokines or induce a febrile response (16;19;24). Other *in vivo* models report that oat derived beta glucan can prevent increased risk of URTI as a result of stressful exercise in mice (6;14). A possible mechanism might be that beta glucan boosted the nonspecific host immune response to infection. Currently, the exact mechanism of action is unknown (10).

Beta glucan helps to reduce post-surgical infections and reduce intensive care unit stay length (1;2;7). Other dietary supplements may reduce URTI symptoms (20), for example zinc treatment reduced severity and duration of cold symptoms (21). A recent study reported no change in self-reported URTI symptoms in endurance athletes given a beta glucan supplement for 18 days (18). They also reported no changes in natural killer cell activity, polymorphonuclear respiratory burst activity, phytohemagglutinin-stimulated lymphocyte proliferation, plasma interleukin 6 (IL-6), IL-10, IL-1 receptor agonist (IL-1ra), and IL-8, and blood leukocyte IL-10, IL-8, and IL-1ra mRNA expression. Possible explanations of the lack of association of beta glucan supplements on URTI symptoms may be that the small sample sizes (n = 19 for both) of these studies were too small to detect subtle effects. Also the chemical composition of the supplements may be different from those reported to reduce post-surgical infections. Nieman et al (18) used a 600 mL beverage supplement that was a mix of Gatorade and 5.6 g/day of Oatvantage, a 54% beta glucan concentrate, whereas the current study used 500 mg beta glucan supplement in capsule form. The chemical composition and dosage were different between the two studies, which makes direct comparison difficult. Higher doses of beta glucan may be required to see any effects, such as those reported in rodent studies.

A beta glucan antioxidant supplement may help to suppress symptoms of URTI and increase perceptions of overall health in WLFF during 14 days of arduous wildfire management. This could be important for crew morale. Hand crews are together for 24 hours a day for 14–21 straight days. Morale can be critical in maintaining a positive work environment. While the present study did not provide biological data on the efficacy of the beta glucan supplement on immune system function; beta glucan may decrease URTI symptoms and promote feelings of perceived overall health. However, when asked their overall perception at the end of the study WLFF could not distinguish between the beta glucan phase and the placebo phase. This finding seems somewhat contradictory, but might be due to the fact that the end of the treatment period was often in conjunction with the end of a fire assignment. Most WLFF feel the same at the end of most assignments, tired and worn down. We believe the lack of a difference is due to the nature of the job and not the study. The more important finding is that during the assignment WLFF felt better during the beta glucan treatment. Further research, including larger sample sizes and a range of beta glucan doses, is needed.

Wildland firefighters are endurance athletes based on their long duration work shifts, and total

energy expenditure (23). They must be able to maintain light work intensity for many hours a day on consecutive days, and also be dynamic in the event of an emergency situation. If they are unable to perform their assigned tasks, this can lead to decreased safety and work productivity. Activity data collected from a subset of subjects, demonstrates that activity levels were consistent across conditions, similar to previously reported findings from our laboratory (5). The average counts demonstrated that the average level was light activity (Figure 1). The beta glucan supplement may possibly decrease the incidence of URTI symptoms when activity levels are mostly light and sedentary.

Limitations of this study were the dynamic nature of the occupation and the length of the trial. Crews were not always completing a treatment while on a fire. There were instances when travel days at the beginning of a treatment led to early termination of the 14 day fire assignment. Given that this trial was also only 14 days, future research should determine the summative effects of the supplement over a season of firefighting.

The Beta Glucan supplement may possibly decrease the incidence of URTI symptoms. This study showed a decreased trend for URTI during the active treatment. This supplement could possibly help WLFF combat one of the most common health related problems described by WLFF.

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Field Based Study 5: Effects of Modafinil and sleep loss on physiological parameters.

J.S. Cuddy[1], Andrew R. Reinert[1], Kent C. Hansen[2], and B.C. Ruby[1]

[1]Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

[2]The University of Wisconsin-Madison
Department of Nutritional Sciences
1415 Linden Drive
Madison, WI 53706

Introduction:

Few scenarios in everyday living require sustained human performance lasting longer than 24 hours. However, certain recreational events, such as ultra-endurance running, adventure racing, mountaineering, and long distance cycling/mountain biking, require sustained performance over long periods of time (>24 hours). For warfighters engaged in training or combat, continuous operations demanding high energy expenditures are an everyday reality. Resisting fatigue and extending human performance during extended operations are goals for the modern warfighter.

In recent years Modafinil has been used by the military to improve cognitive performance during extended wakefulness. Modafinil (2-[(diphenyl-methyl)-sulfinyl]acetamide) is a psychostimulant commonly used clinically for narcoleptic patients and has minimal reported side effects[1-4], low abuse potential[4, 5], and proves effective in promoting wakefulness[2, 3, 5-7]. Although the mechanism by which Modafinil works has not been fully elucidated, researchers have proposed it promotes wakefulness by activating α-1 noradrenergic transmission on hypothalamic cells that contain the peptide hypocretin, modulating GABAergic tone, or by inhibition of the dopamine reuptake transporter[4].

While multiple studies have investigated Modafinil and cognitive performance, few studies have evaluated Modafinil's effect on physical performance, thermoregulation, and total energy expenditure (TEE). Past research has alluded that Modafinil may reduce self-selected exercise performance due to the increased thermal strain. However, Jacobs and Bell[8] found that acute Modafinil administration to well rested subjects increased cycling time to exhaustion by 22% compared to placebo at 85% VO_2 max. Heart rate was elevated at all time points and VO_2 ($L \cdot min^{-1}$) was higher at exhaustion. In another study by McClellan et al[9] in which sleep deprivation was present, elevations in heart rate, rectal temperature, and skin temperature during rest and exercise (65% VO_2 max for 2 hr) were observed in response to Modafinil administration. Modafinil has been shown to affect thermoregulation; specifically, it has been shown to increase core body temperature in the range of 0.3 to 0.5°C[9-12] and increase skin temperature[9, 12], thereby suggesting a greater physiological and thermal strain.

Previous military research has shown TEE for combatants ranging from 13.0 to 29.8 $MJ \cdot d^{-1}$, with a mean of 19.3 ± 2.7 $MJ \cdot d^{-1}$ [13]. Military research has used the doubly labeled water method extensively for calculating TEE, since it is the most practical technique to use during dynamic and free living activities outside of laboratory settings[14-19]. Modafinil has been shown to improve physical performance[8], but its effect on the measure of TEE during free-living military training has not been evaluated. Since TEE cannot accurately ascertain the complete physiological strain a person undergoes, total body water turnover (rH_2O) has been used alongside TEE to demonstrate work stress[20]. Higher total body water turnover can be accounted for by higher sweat rate in exercising individuals compared to non-exercising controls, which may promote a greater rate of fluid intake[21, 22].

Special Operations Operators' training involves a multitude of different physical and mental tasks, often requiring periods of sleep deprivation. These operators must possess the ability to physically perform when extremely fatigued and sleep deprived, and must maintain alertness for extended operations. Although wakefulness drugs, such as Modafinil, are frequently used for sustaining wakefulness, the effects of this drug during sleep deprivation on physical performance, thermoregulation, and TEE have been minimally evaluated.

The purpose of this study was to determine the effects of Modafinil administration on measures of physical performance, thermoregulation, and TEE during continued wakefulness (72 hours) in Air Force Special Operations operators. Based on past research, we hypothesized that the group taking Modafinil would perform better on the physical tests, have increased thermal strain, and have higher energy expenditure.

Methods:

Participants

Study participants included twelve male Air Force Special Operations Pararescue Jumpers and Combat Controllers. Study participants were briefed regarding the risks involved with participating in the study and completed a University Institutional Review Board approved consent form.

Procedures:

Modafinil Dosing
Subjects stayed awake for all but two hours of the 72 hour study. The two hours of sleep were between 0820 and 1020 on Day 3. Subjects were randomly assigned treatment and placebo groups, and the capsules were administered in a double blind fashion. For dosing amounts and schedule, see Figure 1. Subjects were allowed to consume food and fluids *ad-libitum.*

Figure 1. Modafinil dosing, urine collection, and physical testing (PT) schedule.

Time	Day 1	Day 2	Day 3	Day 4
0000		100 mg dose	200 mg dose	200 mg dose
0700	Urine	Urine	Urine	Urine
0800		100 mg dose	100 mg dose	
1600		100 mg dose	100 mg dose	
1800	PT Test	PT Test	PT Test	
2000		Urine	Urine	

Tracer Ingestion and Urine Collection

The night prior to commencement of the study, subjects ingested an oral dose of $^{2}H_2O$ (0.23 $g \cdot kg^{-1}$ estimated total body water) and $H_2{}^{18}O$ (0.39 $g \cdot kg^{-1}$ estimated total body water). Overnight and first void urine volume was collected the first morning. Daily second void urine samples were collected between 0500 and 0700. Additional samples were collected daily between 2000 and 2100. All subjects had been living in the Florida environment for at least six months, so the collection of control samples to adjust for shifts in background isotope enrichment was unnecessary.

Performance Tests

Every evening, operators completed the Special Tactics Physical Performance Test for Air Force Special Operations operators. The complete test has five different components: maximum pushups in two minutes, maximum sit-ups in two minutes, maximum pull-ups (no time limit), three mile run as quickly as possible, and a one mile swim in a twenty-five yard pool as fast as possible wearing a mask, snorkel, and fins. Subjects were given a standardized amount of time in between tests.

Measurements:

Performance Tests

Operators were provided detailed instruction of proper form for each exercise. Participants paired together and counted each other's repetitions for the pushups, sit-ups, and pull-ups. When time ended or the operator participating in the test reached volitional exhaustion, his partner immediately recorded the number of repetitions. For the three mile run, trained researchers were positioned at the halfway point and the finish line to record times with stopwatches. Researchers counted laps and recorded times with stopwatches for the one mile swim.

Oral temperature

Oral temperature was taken each day at 0500, 1100, 1600, and 2300 using a calibrated digital thermometer. One subject was dropped from the analysis due to sample contamination at one time point.

Isotopic analysis

Urine samples were mixed with 200 mg dry carbon black (Fischer Scientific Chemical Co., Itask, IL, USA) and filtered (0.45 µm acetate, GE Water & Process Technologies, Minnetonka, MN, USA). For deuterium analysis, 1ml of the cleaned urine sample was transferred into a vial (Target I-D™ Vials, National Scientific Company, Lawrence, GA, USA) and sealed. An autosampler injected 0.8µl of sample into a quartz tube containing chromium metal powder (Mesh size 100 and finer, Fischer Scientific Chemical Co., Itaska, IL, USA) to reduce water to hydrogen gas[23]. Deuterium abundance was measured using a Finnigan MAT Delta Plus isotope ratio mass spectrometer (Thermo Finnigan, San Jose, CA). Samples were injected in duplicate and all participants' samples were analyzed during the same batch. The analyses were corrected for H^+_3 as well as memory from the reduction system. The standard deviations for duplicate deuterium analyses were 0.6 and 1.0 permil at low and high abundances, respectively. For ^{18}O analysis, a second 1ml aliquot of cleaned urine was transferred to a Vacutainer (3ml, Becton Dickinson and Co., Franklin Lakes, NJ, USA) containing 1ml CO_2 (STP) and equilibrated for at least 24h at 30°C[24]. Approximately 15µl of equilibrated CO_2 was chromatographed to separate it from air and introduced into a continuous flow inlet system using helium as a carrier gas and analyzed on a Delta-S isotope ratio mass spectrometer (Finnigan MAT, San Jose, CA, USA)[24]. Analyses were performed in duplicate and all participants' samples were analyzed during the same batch. The standard deviations for duplicate oxygen-18 analyses were 0.17 and 0.4 permil at low and high abundances, respectively.

Total Body Water (TBW) Calculation

Isotope dilution spaces were determined using the urinary enrichment above baseline of each isotope at 6- and 7-h post-dose. Isotope dilution spaces (N, kg) were calculated according to Cole and Coward[25],

$$N = (WA/1000a)(d_a-d_t)/(d_s-d_p)-w,$$

where W is the grams of water used in the dilution of the dose water, A is the grams of dose water used in the dilution, d is the per mil isotopic abundance of the diluted dose water (d_a), the tap water used in the dilution (d_t), the post-dose urine specimen (d_s), the pre-dose urine specimen (d_p), and w is the amount of water (kg) consumed between the DLW dose and the second (8h) post-dose urine specimen. TBW was calculated from the average of the deuterium and oxygen dilution space divided by 1.041 and 1.007, respectively, to correct for in vivo isotopic exchange[26].

Total Energy Expenditure

Using the slope/intercept method, the ^{18}O and deuterium elimination rates (k_o and k_d) were calculated from the change in the natural logarithm of isotope enrichment as a function of time elapsed after dose administration. The enrichments at equilibration and subsequent daily urine samples through 3 days of isotope elimination and the actual time elapsed were used in calculating the elimination rates. Carbon dioxide production was calculated according to Racette et al[26],

$rCO2 = 0.455TBW(1.07k_O - 1.041k_H)$,

where TBW is the total body water in moles, k_O and k_H are the oxygen and deuterium elimination rate in pools/day, respectively. From rCO_2 TEE was calculated using the modified Weir equation[27] using a respiratory quotient of 0.85 for a mixed diet. Energy expenditure from activity (EEA) was calculated based on procedures used by Ruby et al[28].

Statistical Procedures

All descriptive data are expressed as means ± SD. Independent 2-tailed t-tests were used to determine differences between groups for subject characteristics and rH_2O. Two-factor mixed design analysis of variance for treatment and time was used to determine differences in performance tests, oral temperature, and energy expenditure between groups. Statistical significance was established using an alpha level of $p<0.05$.

Results:

Subject Characteristics

Subject descriptive data are shown in Table 1. There was a significant main effect of time for body weight, which demonstrated a significant increase from pre to post ($p<0.05$).

Table 1. Subject descriptive data.

Variable	Modafinil	Placebo	Overall Mean
Pre Body Weight (kg)	82.4 ± 8.6	81.6 ± 11.3	82.0 ± 9.6
Post Body Weight (kg)	83.5 ± 9.0	83.2 ± 11.7	83.4 ± 10.0*
Body Fat (%)	14.3 ± 4.0	17.5 ± 5.2	15.9 ± 4.7
TBW (kg)	51.4 ± 4.6	49.0 ± 5.8	50.2 ± 5.2
FFM (D_2O dilution – kg)	70.4 ± 6.4	67.1 ± 7.9	68.8 ± 7.1
FM (D_2O dilution – kg)	12.0 ± 4.1	14.5 ± 5.5	13.2 ± 4.8

Values are reported as mean ± standard deviation. *$p<0.05$ versus pre, main effect of time. n=12. TBW = total body water, FFM = fat-free mass, FM = fat mass.

Performance Tests

There were no significant differences between the Modafinil and placebo groups for pushups, sit-ups, three mile run, or the one mile swim (Table 2). There were also no differences in these performance measures over time. Although there were no differences between groups, the maximal number of pull-ups was higher on day one compared to days two and three ($p<0.05$).

Table 2. Physical performance tests.

Variable	Day 1	Day 2	Day 3	Overall Mean
Pushups	85.4 ± 2.4	85.7 ± 1.5	84.3 ± 5.1	85.1 ± 3.3
Modafinil, Placebo	85.2, 85.5	85.2, 86.2	84.2, 84.3	
Sit-ups	114.1 ± 20.3	104.4 ± 9.1	104.5 ± 18.7	107.7 ± 16.9
Modafinil, Placebo	121.6, 107.8	108.4, 101.0	108.8, 101.0	
Pull-ups	17.1 ± 5.7	13.6 ± 2.5*	13.5 ± 4.1*	14.7 ± 4.5
Modafinil, Placebo	16.2, 18.0	13.2, 14.0	13.2, 13.8	
Three Mile Run	22.1 ± 1.3	22.8 ± 1.8	22.7 ± 1.6	22.6 ± 1.6
Modafinil, Placebo	21.4, 22.7	21.9, 23.8	22.0, 23.4	
One Mile Swim	26.2 ± 3.0	26.4 ± 2.5	26.8 ± 3.0	26.4 ± 2.7
Modafinil, Placebo	26.4, 26.1	26.5, 26.3	26.3, 26.8	

Values are reported as mean ± standard deviation. *p<0.05 versus day one, main effect of time. n=11

Oral Temperature

For time points following Modafinil ingestion, there was a significant main effect for treatment, with the Modafinil group having oral temperatures higher than the placebo group (36.5 ± 0.2°C and 36.3 ± 0.2°C for the Modafinil and Placebo groups, respectively, p<0.05, Fig. 2). One subject had to be dropped from this analysis due to a poor reading at one time point.

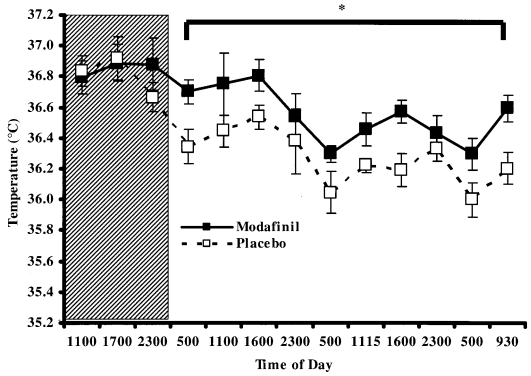

Figure 2. Oral temperature measures over 72 hours of continued wakefulness. The shaded areas indicate the time prior to Modafinil ingestion. *p<0.05 between Modafinil and placebo group, main effect for treatment.

Water Turnover

Data for rH_2O are presented in Table 3; there were no overall differences between the Modafinil and placebo groups.

Table 3. Energy expenditure and total body water turnover.

Variable	Modafinil	Placebo	Overall Mean
TEE ($MJ \cdot d^{-1}$)	19.4 ± 3.7	19.9 ± 2.1	19.7 ± 2.9
($kCal \cdot d^{-1}$)	4646 ± 877	4765 ± 499	4706 ± 684
TEE ($kJ \cdot kg^{-1} \cdot d^{-1}$)	235.1 ± 31.8	245.6 ± 13.4	240.6 ± 23.8
($kCal \cdot kg^{-1} \cdot d^{-1}$)	56.2 ± 7.6	58.7 ± 3.2	57.5 ± 5.7
EEA ($MJ \cdot d^{-1}$)	9.6 ± 2.9	10.3 ± 1.3	10.0 ± 2.2
($kCal \cdot d^{-1}$)	2290 ± 700	2470 ± 317	2380 ± 526
rH_2O ($L \cdot d^{-1}$)	8.8 ± 1.0	9.0 ± 1.5	8.9 ± 1.3
rH_2O ($ml \cdot kg^{-1} \cdot d^{-1}$)	106.5 ± 10.4	110.4 ± 13.9	108.5 ± 11.9

Values are reported as mean ± standard deviation. n=12. TEE = total energy expenditure, EEA = energy expenditure from activity, rH_2O = total body water turnover.

Energy Expenditure

There was a significant main effect of time for total energy expenditure (TEE) and energy expenditure from activity (EEA) ($p<0.05$). TEE was higher on day one compared to days two and three [23.8 ± 4.8, 18.2 ± 3.3, and 16.8 ± 3.5 MJ (5686 ± 1158, 4359 ± 791, and 4005 ± 836 $kCal \cdot d^{-1}$) for days one, two, and three, respectively, $p<0.05$, Fig. 3]. EEA was higher on day one compared to days two and three [13.6 ± 3.9, 8.7 ± 2.9, and 7.3 ± 3.1 MJ (3262 ± 931, 2068 ± 682, and 1749 ± 730 $kCal \cdot d^{-1}$) for days one, two, and three, respectively, $p<0.05$, Fig. 3]. There were no overall differences between the Modafinil and placebo groups.

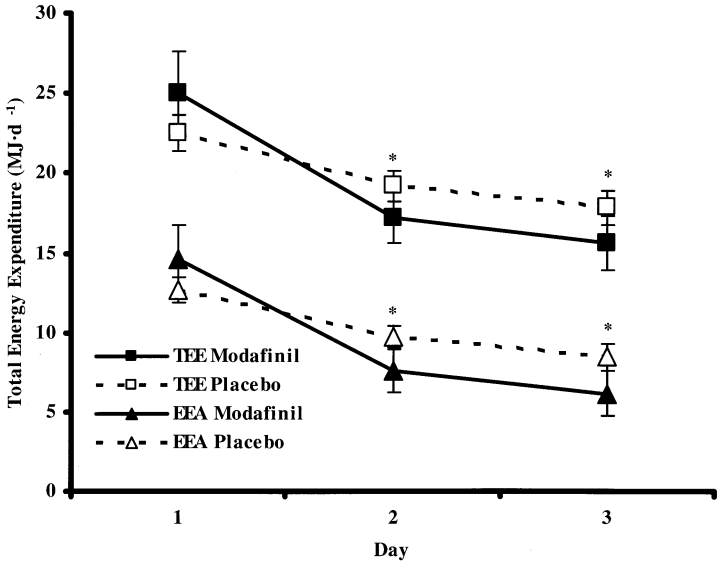

Figure 3. Daily total energy expenditure (TEE) and energy expenditure from activity (EEA). $MJ \cdot d^{-1}$ for the Modafinil and placebo groups is displayed. *$p<0.05$ between day one and days

two and three, main effect for time.

Discussion:

Understanding the physiological requirements of occupational demands, and whether they change with drug administration, is critical to maximize performance, train appropriately, and to best maintain adequate dietary intake. In this study there were no differences between Modafinil and Placebo groups for physical performance, rates of water turnover (rH_2O), and total energy expenditure (TEE), despite the Modafinil group having significantly higher oral temperatures. It appears that during extended wakefulness (72 hours), Modafinil provides no benefit for physical performance and the drug does not alter total body water turnover despite a subtle, yet significantly increased oral temperature.

Physical performance

After two and three days of wakefulness operators retained the physical stamina to complete physical tasks independent of Modafinil ingestion, particularly endurance tasks (5 kilometer run and 1.6 kilometer swim). It is interesting that despite having been awake for ~36 and ~60 hours (the times the performance tests occurred) the operators maintained similar performances to the first testing session at ~10 hours into the study. This was also the case for the placebo group, which is in contrast to previous research that has demonstrated a decline in endurance performance following sleep deprivation[29]. The current findings are in contrast to Jacobs and Bell[8], who found a performance benefit in time to exhaustion with Modafinil. There is the possibility that the placebo group was superior in physical fitness compared to the Modafinil group, but times during the run and swim were similar during all three days. Another possible explanation for these findings might be the strong mental disposition these individuals possess. Special Forces Operators are mentally strong individuals, and their mental drive might have overridden any effects of fatigue more prevalent in recreationally active subjects.

Perhaps the Modafinil doses were not high enough to have an ergogenic effect for any of the physical tests in this study. In the study by Jacobs and Bell[8] in which acute Modafinil ingestion increased time to exhaustion, subjects were administered a 4 $mg \cdot kg^{-1}$ dose, or an absolute dose in the range of 262 to 382 mg. They then completed their exercise test three hours after drug administration. In the current study, at the time of commencing the physical tests, participants had ingested only 0.8 $mg \cdot kg^{-1}$ (an absolute dose of 100 mg) two hours prior to the PT tests. This was 80% less than the amount provided by Jacobs and Bell[8]. While varying levels of Modafinil ingestion have not been tested for a dose-dependent effect on physical performance, Wesensten et al. suggest a trend toward better cognitive performance in a dose-dependent effect, despite finding no significant differences in cognitive performance among 100 mg, 200 mg, and 400 mg doses of Modafinil[2]. While it is inconclusive whether there is a dose dependent effect (and whether that would even translate to improved physical performance), perhaps the doses in the current study were too small to impact the subjects because of their high fitness levels and high amount of fat-free mass. However, Baranski et al.[30] have demonstrated that the dosing regimen used in the current study is effective for preventing a decline in fatigue and cognitive performance during sustained wakefulness. Thus, our dosing regimen, while possibly on the

lower end compared to other studies[2, 8], has been proven to provide benefit for increased cognitive performance and reduced fatigue.

Thermoregulation

In the current study, the group receiving Modafinil had an overall average oral temperature that was 0.3°C higher compared to the placebo group (Figure 2). Pigeau et al.[11] did not find any differences in oral temperature due to large inter- and intra- subject variability, but did find increased core body temperature with Modafinil ingestion. Other studies using Modafinil have demonstrated increases in core temperature in the magnitude of 0.3 to 0.5°C[9-12], similar to the present investigation. While it has been suggested that the thermogenic property of Modafinil might impede physical performance by predisposing individuals to heat strain[31], this study found that performance during physical tasks was not affected. While Modafinil has consistently shown minor increases in core and/or oral body temperature, this increase has negligible physiological effects that hinder physical performance. Had thermal strain increased substantially and caused increased sweat rates, subjects would have demonstrated higher water turnover in an effort to remove heat from the core via sweating (a function of increased fluid intake and loss)[21, 22]. However, rH_2O values for the present study were 107 ± 10 $mL \cdot kg^{-1} \cdot 24hr^{-1}$ and 110 ± 14 $mL \cdot kg^{-1} \cdot 24hr^{-1}$ for the Modafinil and placebo groups, respectively.

Total energy expenditure

The average daily total energy expenditure (TEE) during the current study was 19.7 ± 2.9 $MJ \cdot d^{-1}$, similar to the mean of 19.3 ± 2.7 $MJ \cdot d^{-1}$ found in previous military research[13]. While the physiological stresses involved in typical military training have been well documented, to our knowledge no study has investigated the effects of Modafinil administration on TEE. Since Modafinil increases wakefulness, we hypothesized that it would contribute to an increase in TEE because operators might maintain higher vigor and possibly stay on task better and self-select more work due to a lack of fatigue. In this study, the group administered Modafinil demonstrated similar values for TEE and energy expenditure from activity (EEA) compared to the placebo group. There was a significant main effect of time for the measures of TEE and EEA from day one to days two and three, showing reduced energy expenditure during extended wakefulness in military training (Figure 3), even when the scripted activities were similar each day.

If a wakefulness drug such as Modafinil did increase TEE and rH_2O, operators would require additional fluids and supplemental foods during extended operations. These findings suggest that it is unnecessary for individuals taking Modafinil to carry additional fluids and/or food. A limitation of this study was that daily garrison activities were scripted by the researchers, limiting the opportunity for altered self-selected work by the study participants. Although the TEE between groups was determined, it is not possible to distinguish the effects of Modafinil on self-selected work activity during extended wakefulness. An area for future research should include the effects of Modafinil on activity patterns when self-selected work can be varied. This approach would also help elucidate the possibility of unique nutritional/hydration requirements during non-scripted garrison/training/operational tasks.

Conclusions:

Operators retained the physical stamina to complete physical tasks independent of Modafinil ingestion, particularly for tasks requiring muscular endurance. While incurring a higher oral temperature compared to placebo, rH_2O was similar between the Modafinil and placebo groups. Additionally, Modafinil did not elevate TEE compared to placebo. Further research should compare Modafinil to placebo and/or other wakefulness drugs in sleep-deprived environments where subjects can better self-select the intensity of their work activity extended operations. Additionally, determining whether a dose-dependent effect of Modafinil ingestion exists for improving physical performance warrants future investigation.

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Field Based Study 6: Accelerometry and Corresponding Physiological Stress During Air Force Special Tactics Officer Selection.

J.S. Cuddy[1], A.R. Reinert[2], W. Hailes[1], D.R. Slivka[1], J.K.Wright[2], and B.C. Ruby[1]

[1]Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

720th Special Tactics Group
U.S. Air Force Special Operations Command
Hurlburt Field, FL
32544

Introduction:

United States Air Force Special Tactics Officer (STO) Selection is conducted biannually in an effort to select officers who possess the necessary leadership qualities to enter the combat controller training pipeline. Air Force Special Tactics operators typically work in close cooperation with other special operations units. As a special operations ground combatant force, they maintain the same or higher physical attributes of strength, stamina, and endurance as other elite Special Operations Forces (SOF). Identifying selection measures of SOF combatants is complex, but physical prowess, motivation, and spatial ability have been recognized as key factors[1]. Additionally, officers require strong leadership skills, and the ability to think clearly during stressful, ambiguous situations.

While many stressors involved in STO Selection are physical (ruck marching, pool sessions, sleep deprivation, limited caloric intake, etc.), a considerable component of the selection process is related to qualities of mental resiliency that candidates exhibit during confusing and stressful circumstances. Candidates problem solve and work together to achieve specific outcomes, and typically these are unreasonable situations that do not have a solution. Collectively, the physical and mental components of STO Selection combine to increase the overall stress load on candidates during the week long selection process.

When exposed to acute physical and/or mental stresses, a cascade of multiple hormones, including cortisol, prepares a person for physical movement and/or protection. Cortisol is a glucocorticoid that assists in partial regulation of carbohydrate, fat, and protein metabolism, and can be used as an acute and chronic indicator of stress[2]. While diurnal fluctuation in cortisol is normal[3], an acute increase in cortisol can be caused by both psychological[4-7] and physiological[8-10] stimuli. In addition, cortisol responds to changes in training status and performance during the course of a sport season[9]. In several studies observing stress responses to simulated prisoner of war camps, Morgan et al. found acute increases in cortisol in response to stressors placed upon participants[11-14]. Cortisol increased in response to survival training stress[13] and high cortisol was associated with greater subjective distress[11], greater dissociation during[12] and following stress[14], and reduced military performance[12]. In elite golfers cortisol levels increased prior to competition,

but there was no relationship between cortisol levels and performance[15]. In contrast, during weightlifting competition an acute influx of cortisol prior to competition was beneficial for improving performance[16]. It is difficult to ascertain whether increased cortisol prior to competition always improves performance[17-19], but the heightened state of being, "fight or flight," prepares athletes for competition. Thus, it is unclear whether elevated cortisol levels are beneficial or detrimental to physical and/or mental performance during stressful situations.

Although past research has established the typical energy expenditure[20] for military operations, descriptions of daily activity patterns have been limited[21-23]. Activity monitors can estimate energy expenditure and quantify physical activity patterns, making them a practical, simple, non-invasive research tool. The use of monitors for tracking activity has been used in diverse subject populations[21-27]. A primary advantage of using activity monitoring is the ability to classify activity into different metabolic intensities, revealing how hard participants work. Analyzing alterations of cortisol alongside activity data may provide indicators of candidates' resiliency to stressful situations.

The aims of the current study were to establish physical activity patterns, estimate energy expenditure, and identify whether return and/or successful candidates demonstrated differences in cortisol responses compared to non-selected and/or first time attendees.

Methods:

The research study was approved by the Institutional Review Boards at The University of Montana (Protocol #70-07) and Air Force Research Laboratories, Wright Site Institutional Review Board Protocol # F-WR-2007-0032-H). Prior to beginning the study, researchers briefed participants on the requirements for being a subject, and made clear that participation in this study would in no way affect the outcome of STO selection. Participation in the study was voluntary, and subjects provided written informed consent. Data collection took place at Hurlburt Field, FL.

Subjects

Subjects included candidates (n=11, mass 76 ± 6 kg, height 177 ± 9 cm, and age 26 ± 3 y) striving to become Special Tactics Officers. One subject was unable to pass the initial PT tests and was eliminated the first night of the study. Two subjects voluntarily eliminated themselves from the selection process on Day 3 of the study. Two candidates were unable to complete the final event due to injury. Activity data represents the six participants who completed the entire selection process, and salivary cortisol represents nine participants through Day 3, and seven participants through Day 5. One first time non-selected candidate had to be dropped from cortisol analysis due to sample contamination at one time point.

Experimental Design

Candidates participated in a wide range of different activities during the five day selection process, including: running, swimming, calisthenics, ruck marching, water skill sessions, leadership reaction courses, and "Monster Mash" (a several hour mission that included swimming, land navigation, ruck marching, load carrying, and skill tests). A specific time frame

of events is not available for public distribution due to the need for the course to remain unpredictable and ambiguous for future candidates. During the STO Selection course, subjects participated in the following: 1) pre and post body weight, 2) measurement of activity patterns by wearing ActiCal® activity monitors on the wrist, and 3) saliva samples were collected at eleven time points to assess changes in cortisol.

Body Weight and Height
Subjects' body weight was measured using a digital scale (Detecto, Model-758C, Webb City, MO) at the beginning and end of the study. Height was measured using a stadiometer. For all measurements, subjects wore socks and training shorts.

Physical Activity Patterns and Total Energy Expenditure
Activity was measured by placement of a small ActiCal® activity monitor (MiniMitter, Bend, Oregon) with an adjustable hospital band on the non-dominant wrist with the blue arrow pointing toward the elbow[28]. The wrist location has been previously used with accelerometry measurement during military research[21-23]. The monitor was worn continually for the five day study. Daily energy expenditure was calculated indirectly by using ActiCal® 2.0 software (Bend, OR), and task specific energy expenditure was estimated using previously established algorithms[28]. Energy expenditure for the run portion of the study was calculated using the American College of Sports Medicine (ACSM) running metabolic equation[29], and energy expenditure for the swim portion was calculated using the *Compendium of Physical Activities*[30]. Activity intensity was classified based upon the following cut points: sedentary and light (0-144 $kcals \cdot hr^{-1}$), moderate (145-386 $kcals \cdot hr^{-1}$), and vigorous (387+ $kcals \cdot hr^{-1}$)[28]. To discriminate between energy expenditure in the sedentary and light categories, activity monitor cut points were used: sedentary (0-50 $counts \cdot min^{-1}$), and light (50-600 $counts \cdot min^{-1}$).

Salivary Cortisol
Saliva was collected (~3 ml) by passive drool and frozen at -30º C. Salivary cortisol was measured using a competitive immunoassay on a micro-plate reader (Model 680 XR, Bio-Rad, Hercules, CA) at 450 nm in accordance with the manufacture's protocol (Salimetrics, State College, PA).

Data Analysis
All descriptive data was expressed as means ± standard deviation. A dependent t-test was used to determine differences in body weight. To determine whether daily stressors elevated cortisol and to compare cortisol data between return versus first time candidates and selected versus non-selected, a repeated measures analysis of variance for group by time was used. Statistical significance was established using an alpha level of $p<0.05$.

Results:

Body Weight, Height, and Age
There was no significant change in body weight from Day 1 to Day 5 (77.8 ± 3.1 kg and 77.6 ± 3.2 kg for pre and post, respectively). There were no differences in body weight, height, and age between the first time and return candidates.

Activity Data

The average daily activity counts for the six subjects who completed the entire selection process were 684 ± 200 counts·min^{-1}. There was a significant decline in activity from Day 1 to Days 2, 3, 4, and 5, $p<0.05$ (Figure 1). Day 4 was lower than Day 3 ($p<0.05$). Time spent in different intensities and task specific activity counts are detailed in Tables 1 and 2. Too few participants completed the "Monster Mash" on Day 5 (n=6, only one candidate who was a return) to statistically compare between groups for activity and energy expenditure. Non-statistically, after three days first time candidates had fewer counts·min^{-1} compared to return candidates (746 ± 114 and 857 ± 29 counts·min^{-1}, respectively).

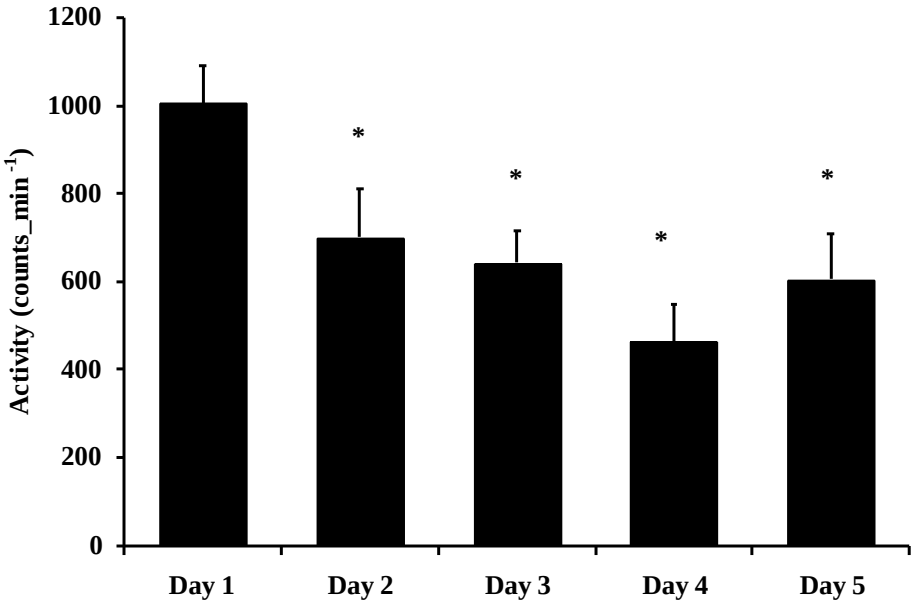

Figure 1. Activity counts during STO selection (n=6). Values are expressed as mean ± SD. * $p<0.05$ compared to Day 1. † $p<0.05$ compared to Day 3.

Table 1. Activity intensity profile STO selection. Values are expressed as mean ± SD.

Intensity	n	Duration ($hr \cdot day^{-1}$)	Estimated Energy Expenditure ($kcals \cdot day^{-1}$)	Activity ($counts \cdot min^{-1}$)	% of Total Time
Sedentary	6	8.7 ± 2.0	652 ± 142	16 ± 4	36 ± 8
Light	6	9.3 ± 1.4	1483 ± 214	304 ± 14	39 ± 6
Moderate	6	4.7 ± 0.5	1362 ± 158	1493 ± 79	20 ± 2
Vigorous	6	1.2 ± 0.5	608 ± 326	5580 ± 1122	5 ± 2

Table 2. Task specific activity counts, duration, and energy expenditures. * Kcals derived from [29]. †Kcals derived from [30]. Values are expressed as mean ± SD.

Activity Data	n	Sessions	Activity ($counts \cdot min^{-1}$)	Duration ($min \cdot session^{-1}$)	Estimated Energy Expenditure ($kcals \cdot session^{-1}$)
PT Test Calisthenics	10	1	2145 ± 232	31 ± 0	123 ± 15
PT Test Run	10	1	13,127 ± 2736	22 ± 1	392 ± 30*
PT Test Swim	10	1	5082 ± 1256	29 ± 3	384 ± 38†
Ruck Marching	9	3	1940 ± 350	193 ± 36	769 ± 119
Pool Sessions	7	2	1389 ± 882	183 ± 7	584 ± 84
LRC	9	2	565 ± 153	246 ± 37	600 ± 116
Night LRC	7	1	790 ± 160	369 ± 0	1062 ± 88
Monster Mash	6	1	1789 ± 627	293 ± 0	1155 ± 186

Energy Expenditure

The average daily estimated energy expenditure for the 6 subjects who completed the entire selection process was 4105 ± 451 $kcals \cdot day^{-1}$. There was a significant decline in energy expenditure from Day 1 to Days 2, 3, 4, and 5, ($p<0.05$, Figure 2). Day 4 was significantly lower than Day 3 ($p<0.05$). Energy expenditure associated with specific tasks is detailed in Table 2.

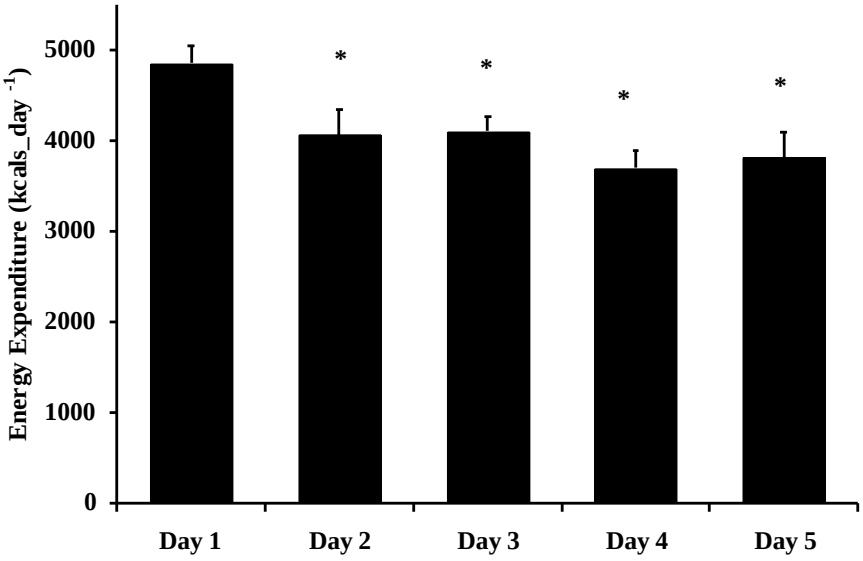

Figure 2. Estimated daily energy expenditure during STO selection (n=6). Values are expressed as mean ± SD. * $p<0.05$ compared to Day 1. † $p<0.05$ compared to Day 3.

Salivary Cortisol and Activity

Salivary cortisol was elevated ($p<0.05$) compared to baseline following four to five hour physical training sessions (time points three, five, and seven), but recovered to similar levels as baseline when candidates were provided times of reduced activity (Figure 3).

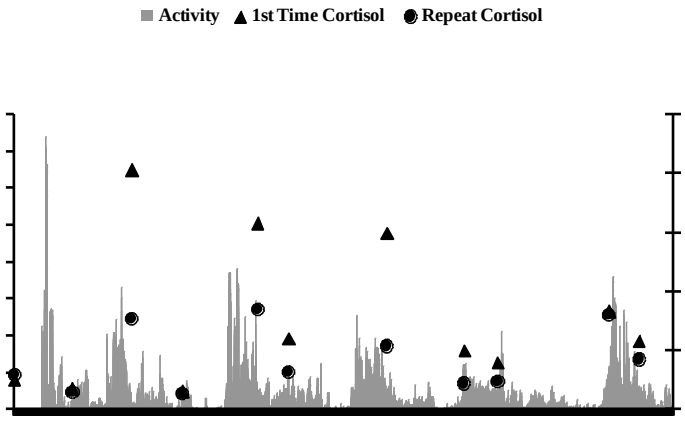

Figure 3. Activity and salivary cortisol during STO selection. * $p<0.05$ compared to baseline (Day 1, 13:00).

Group Differences

First time candidates (n=6) had higher cortisol than return candidates (n=3) (0.90 ± 0.22 ug·dl^{-1} versus 0.43 ± 0.13 ug·dl^{-1}, respectively, $p<0.05$, Figure 4) during the first three days (time points 1-7). After two candidates withdrew on Day 3, four first-time candidates remained. Since three days of data does not address the entire five day selection process, the three returnees were compared to the four first-timers for the entire selection process. When the four first time candidates were compared to the three return candidates for five days, there was still a lower cortisol response for those who had previously attended, 0.43 ± 0.06 ug·dl^{-1} compared to 0.76 ± 0.18 ug·dl^{-1} for first-timers, $p<0.05$. There was a trend toward a difference between selected and non-selected candidates for cortisol, (0.56 ± 0.27 ug·dl^{-1} and 0.89 ± 0.25 ug·dl^{-1}, respectively, $p=0.09$).

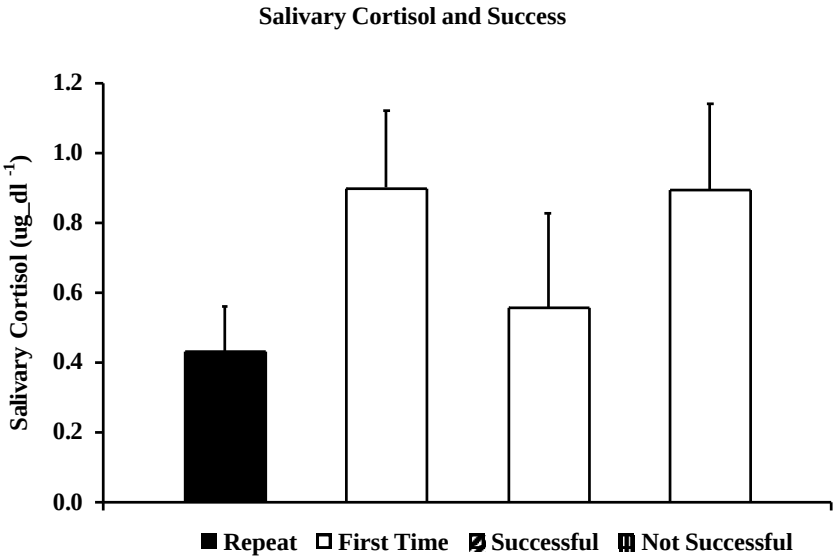

Figure 4. Mean salivary cortisol during the first 3 days of STO Selection for return (n=3), first time (n=6), successful (n=4), and not successful (n=5) candidates. Values are expressed as mean ± SD.

Discussion:

Special Tactics Officer Selection is physically and mentally demanding, with estimated energy expenditures of 4105 ± 451 kcals·day^{-1}. Candidates appeared to do an adequate job maintaining fluid and energy balance, demonstrated by no change in body weight over the five day selection process. The activity data contribute to a growing body of military research using accelerometry as a way to assess activity patterns during sustained military operations[21-23]. Activity patterns are important because they can show work: rest cycles with high resolution (minute by minute), as well as intensity of activity, over an extended period of time (up to 44 days for the ActiCal®). The primary finding in this study was that return candidates had a reduced cortisol response compared to first time participants despite similarities in the estimated total energy expenditure (TEE) and non-statistical evidence that activity was higher for return candidates.

Energy Expenditure

The reduction in energy expenditure over the course of the study suggests that STO selection became progressively less demanding physically. The estimated energy expenditure in this study (4105 ± 451 kcals·day^{-1}) was similar to other military scenarios, which average 4610 ± 650 kcals·day^{-1} [20]. However, these studies have an average time frame of 12.2 days, whereas the current study was five days[20]. Previous research completed during a 10-day Marine Officer Selection Course showed a mean daily energy expenditure of 5378 kcals·day (66.4 kcals·kg^{-1})$^{-1}$ [31] (calculated from doubly labeled water). In contrast, the present 5-day mean estimate for STO selection TEE was 53 ± 6 kcal·kg^{-1}, 25% lower than the Marine Corps study. The ActiCal® does not account for load carriage (a substantial component of STO selection) when estimating energy expenditure, and since load carriage increases energy expenditure[32], this error may partially account for the discrepancy between studies. Though non-statistical, there were minimal differences between groups for energy expenditure (5139 ± 342 and 5495 ± 136 kcals·day^{-1} for the first time and return candidates, respectively).

Activity Patterns

Despite a few limitations with the ActiCal® activity monitor, the activity data collected in this study provides high external validity for future STO selection courses as well as other military scenarios when they involve similar demands. STO selection activities are unknown to candidates entering the course, and are subject to change at any time. High levels of physical and mental stamina are necessary for completion of this course, and activity monitoring provides quantifiable data on the physical aspects of STO selection. The bulk of activity during STO selection was light and moderate intensity, collectively making up ~59% of the daily time. Vigorous activity made up ~71 min·day^{-1}. The run, swim, and portions of ruck marching were the most intense physical activities during the 5-day selection process. During each ruck marching session (carrying a ~27 kg pack), each candidate averaged ~5 minutes of counts·min^{-1} similar to those of running, and 15 minutes of counts·min^{-1} similar to swimming. Thus, portions of ruck marching were arduous even though the ActiCal® did not account for load carriage. Future research could determine ActiCal® activity counts and associated energy expenditure during higher intensity activities and loaded carrying, which would provide better resolution for the metabolic intensities of STO selection.

Salivary Cortisol

During the five days of STO selection, there were daily fluctuations in salivary cortisol (Figure 3), and these changes were dictated by the activity pattern prior to the collection point. The extended duration and high intensity nature of the exercise prior to time points three, five, and seven explains the heightened cortisol response[8, 33, 34]. During times of less physical demand salivary cortisol recovered to baseline levels, indicating that candidates were well-stressed from different tasks, but recovered adequately. Though not statistically analyzed, of particular interest are time points ten and eleven, pre and post "Monster Mash." The activity data suggests ruck marching, pool sessions, and the monster mash are similar to one another (1940, 1389, and 1789 counts·min^{-1}). However, the salivary cortisol response to tasks on days one, two, and three (the three highest cortisol data points) is considerably higher than the post "Monster Mash" time point. Prior to the monster mash, candidates had their lowest activity period (298 ± 81 counts·min^{-1}) in the preceding 21 hours, including ~7.5 hours of sleep. This drop in physical

activity provided time for physical recovery, and, combined with possible adaptation to the mental stressors, might have been the reason for a reduced salivary cortisol response[5, 10].

There was a significant difference in the cortisol response between the candidates who had previously attended STO selection compared to the candidates who were attending STO selection for the first time. Average cortisol values were 43% lower for return candidates over five days, suggesting a reduced response to the stressors of STO selection despite possible higher counts·min^{-1} for return candidates. It is difficult to ascertain a specific reason for reduced salivary cortisol levels in the returning candidates. Morgan et al.[14] suggest that SOF soldiers had a rapid release of NPY and norepinephrine and less difference in baseline/recovery cortisol, demonstrating greater tolerance to stressors than other soldiers. These soldiers were characterized by having greater "stress hardiness," and it is likely the return candidates in this study had been toughened by prior exposure to STO selection. Additionally, since the candidates had a general expectation of the course (STO selection is varied for every class), they might have had reduced anticipatory psychological stress[10] or physically prepared themselves more than first time candidates. Thus, since the physical tasks would not stress them as much and their cortisol would return to baseline quicker than first time candidates.

The current findings warrant further investigation. Three of the four candidates selected were return candidates, thus it brings up the question whether reduced cortisol was a trait for return candidates or successful candidates. There was a trend for a reduced cortisol for those who were selected compared to those who were not ($p=0.09$), and future research could expand this question with a larger study. Additionally, return candidates responded differently to stress and were more likely to be selected than first-time candidates. Prior exposure could provide an advantage for those attending for the second time, or selected candidates have certain physiological responses to stress that set them apart from non-selected candidates.

Conclusions:

The energy expenditure during STO Selection was 4105 ± 451 kcals·day^{-1}, and cortisol increased and decreased in concert with activity patterns. Return candidates had a reduced cortisol response compared to first time candidates, which suggests they handled stresses better. The simple measure of accelerometry can provide data to compare activity patterns of future STO selection courses and different military scenarios to one another. An individual's salivary cortisol response to the stresses incurred during STO selection has the potential to be incorporated into the entire picture of a candidate's performance and potential to handle stress.

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Field Based Study 7: Comparison of energy expenditure and activity patterns during Seal Training Adventures Special Operations Forces Academy and U.S. Air Force Special Tactics Officer Selection

J.S. Cuddy and B.C. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

SEAL Training Adventures^{LLC} Special Operations Forces (SOF) Academy serves the purpose of giving candidates an experience similar to being a member of an active duty SOF platoon. Over a six day time period, instructors provide the toughest mental and physical challenges from each SOF military training program: Navy SEAL, Army Rangers, Marine Recon, and Air Force Combat Controllers and Pararescue Jumpers. Participants in the course are exposed to the following challenges throughout the week: open water swims, pool training, heavy ruck marching, mission preparation, survival skills training, beach runs, difficult PT, obstacle courses, tactical shooting, sky diving, basic SCUBA, sleep deprivation, and land navigation. Goals of the Academy are to assist prospective SOF candidates in deciding whether SOF training is the career they want to choose, as well as help participants choose the military branch that fits their primary interests.

Recently, The Montana Center for Work Physiology and Exercise Metabolism completed a study during Air Force Special Tactics Officer (STO) selection that analyzed activity patterns using ActiCal® activity monitors. The purpose of the project was to determine physical activity patterns and estimate energy expenditure during one week of STO Selection. The primary challenge for researchers with a project involving active duty airmen is access to potential subjects and approval from governmental institutional review boards. If a scenario similar to actual SOF training could be found in the civilian world, some technical hurdles could be bypassed by using civilian participants and obtaining university approval. Thus, the purpose of this project was to compare activity patterns of STO Selection to those of SEAL Training Adventures^{LLC} SOF Academy to determine whether they provide similar physical stresses.

Methods:

Prior to participating in the research, participants provided informed consent that was approved by the University Of Montana Institutional Review Board (Protocol #70-07 and #36-08).. Participants included recreationally active males attending camp and completed various activities throughout the week: open water swims, pool training, heavy ruck marching, mission preparation, survival skills training, beach runs, difficult PT, obstacle courses, tactical shooting, sky diving, basic SCUBA, sleep deprivation, and land navigation. Subjects provided verbal estimates of their height and weight. Activity was measured by placement of a small ActiCal®

activity monitor (MiniMitter, Bend, Oregon) on subjects' non-dominant wrist.

Results:

There were no differences in age, height, and weight between the six STO candidates and the ten SOF Academy participants (Table 1). There were no differences ($p<0.05$) between groups for daily estimated energy expenditure (4105 ± 134 and 4005 ± 696 kcals·day^{-1} for STO and SOF, respectively). Total physical activity was similar between groups, with mean daily activity counts of 684 ± 65 and 695 ± 138 counts·min^{-1} for STO and SOF, respectively. Time spent at different intensity levels can be seen in Table II.

Table 1. Subject descriptive data

	STO Selection	SOF Academy
N	6	10
Age (yrs)	26 ± 3	31 ± 10
Height (in)	70 ± 2	70 ± 3
Weight (lbs)	170 ± 7	164 ± 22

Data is represented as mean ± standard deviation.

Table 2. Time spent at different intensity levels.

	STO		SOF	
	Time (min)	Time (%)	Time (min)	Time (%)
Sedentary	523 ± 57	36 ± 4	473 ± 86	33 ± 6
Light	559 ± 58	39 ± 4	640 ± 49 *	44 ± 3 *
Moderate	282 ± 42	20 ± 3	234 ± 47	16 ± 3
Vigorous	71 ± 35	5 ± 2	93 ± 24	6 ± 2

* Difference between STO and SOF for the respective category, $p<0.05$.

Discussion:

The primary finding of this study was that SEAL Training Adventures^{LLC} SOF Academy closely mimics Air Force STO Selection with respect to energy expenditure and physical activity patterns (Figures 1 and 2). Until now, SEAL Training Adventures^{LLC} had no quantifiable data to uphold their position that their training was similar to other SOF training. This report demonstrates similarities, as well as a few minor differences, between the physical activity patterns of six STO candidates and ten SOF academy participants. The results should be seen as an indication of whether the six-day SOF Academy course mimics the five-day STO Selection.. In short, based on activity monitoring and estimated energy expenditure, SOF Academy provides very similar physical stresses as STO Selection. SOF Academy would be an excellent model to conduct physiology research if parallels wanted to be made to STO Selection. Readers should be hesitant to extend the findings to long-term SOF training.

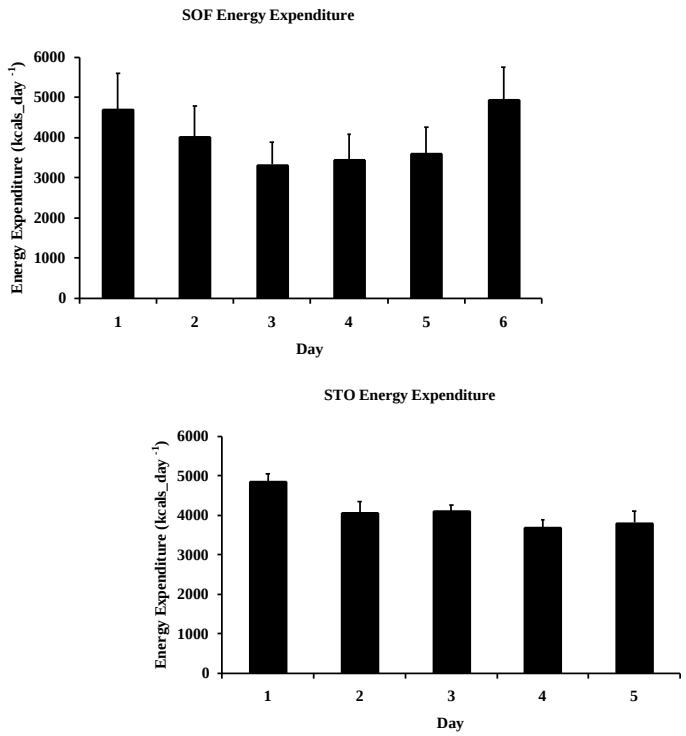

Figure 1. Daily energy expenditure for SOF Academy and STO Selection. There were no differences ($p<0.05$) between groups for daily estimated energy expenditure (4005 ± 696 and 4105 ± 134 kcals·day^{-1} for SOF and STO, respectively).

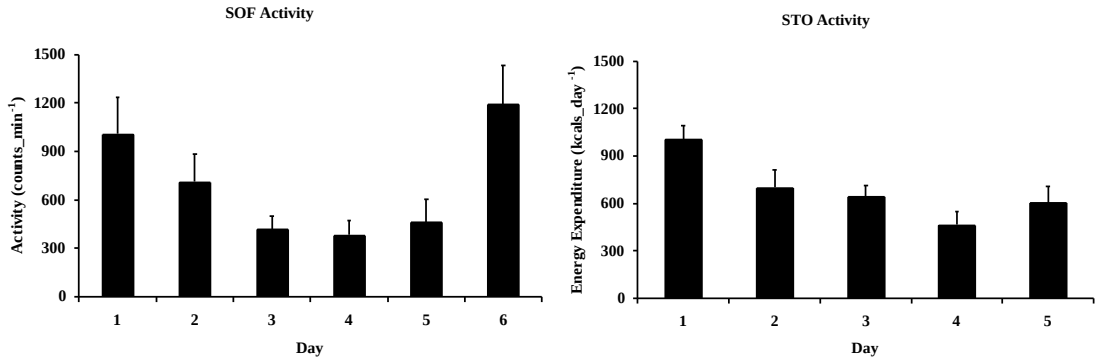

Figure 2. Daily activity patterns for SOF Academy and STO Selection. Total physical activity was similar between groups, with mean daily activity counts of 695 ± 138 and 684 ± 65 counts·min^{-1} for SOF and STO, respectively.

Conclusion:

The six-day SOF Academy course provides physical stress similar to that of the five-day STO Selection program.

Field Based Study 8: Total Energy Expenditure, Energy Substrate Use and Hydration during an Ironman World Championships: A Case Study.

W. Hailes, D. Slivka, C. Dumke, J. Cuddy and B. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

Previous research has measured the total energy expenditure (TEE), race intensities relative to VO_2peak, and muscle glycogen use during a half Ironman triathlon. However the increased demand of a full Ironman distance triathlon puts an unknown additional strain on the metabolism and energy stores. An Ironman triathlon consists of a 3.9 km swim, a 179.2 km cycle, and a 41.9 km run completed as fast as possible and within 17 hours to be an official finisher. An event of this duration and composed of three exercise modalities requires enormous energy expenditure to complete. This energy must come from both endogenous and exogenous sources and be composed of both fat and carbohydrate (CHO) substrates.

Two methods for determining energy expenditure in the field are the doubly labeled water (DLW) technique, and use of regression equations based on the subject's expired gas data. Calculating TEE via use of doubly labeled water provides a gold standard of global metabolic demand of the event, while use of regression equations based on the athlete's expired gas data can estimate the rate of oxidation and relative substrate contribution during the race. The purposes of this study were to measure and compare the TEE using the doubly labeled water and prediction equation methods and to use muscle biopsies to determine the muscle glycogen contribution to overall energy use. The final purpose was to measure the hydration kinetics using water turnover (rH2O) during the Ironman World Championship in Kona, HI.

Method:

Prior to data collection, the protocol was approved by the institutional review board (Protocol # 57-07). The subject was one recreationally trained male from the Missoula community (39 years, 182.9 cm, 77.9 kg, peak VO_2 4.9 $L \bullet min^{-1}$[cycle], 5.4 $L \bullet min^{-1}$[run]). The official race time was used for swimming, running and transition time. The SRM power meter (Schoberer Rad Messtechnik, Welldorf, Germany) was used to measure cycle power output, speed and time. Sub-maximal VO_2 and VCO_2 were used to establish regression equations to estimate the energy use during the cycle and run portions of the race. Energy expenditure during the swim portion was predicted using the equation developed by Kimber and associates.

Linear Regression Equations
Prior to race day the subject ran and cycled at seven sub-maximal intensities on the race course wearing a portable metabolic cart (Cosmed, Rome, Italy). For cycling the sub maximal power output was correlated with the expired gas data to develop an equation that would predict energy

expenditure based on power output, for running pace was used with gas data to develop the appropriate equations.

Water Turnover and TEE
A dose of water that contains a higher than normal amount of a stable isotope for hydrogen (^{2}H) and oxygen (^{18}O) was administered orally after the collection of a background urine sample. After consumption of the original dose mixture, the dose vial was rinsed three times with tap water to ensure complete isotopic delivery (approximately 20 mL per rinse). Subjects were required to refrain from the consumption of food or additional water until first void urine samples the following morning. Second void urine samples were then collected for analysis. After the first void, a nude body mass was obtained (Belfour PS6600T, Saukville, WI, accuracy ± 100 g). All overnight voids were collected to correct the measure of initial TBW. TBW was calculated from the change in hydrogen isotope enrichment (background vs. the second void urine). TEE was calculated by measuring the speed of oxygen isotope elimination (second void urine, pre and post race).

Muscle Biopsy
Two muscle biopsies, pre and post race, were collected from the vastus lateralis muscle of the same leg using a 4 mm Bergstrom percutaneous muscle biopsy needle. After excessive blood, fat and connective tissue were removed the muscle samples were immersed in liquid nitrogen and stored at -80ºC for later analysis.

Results:

The athlete experienced a large flux in body water with a net loss of 5.9 kg body mass, and an estimated 18% plasma volume loss during the race. Despite losing 7.5% of body mass the athlete was able to complete the race. The athlete also experienced increases in hemoglobin concentration and hematocrit in conjunction with the reduction in body mass (table 1).

Table 1. The change from pre to post race in body mass, muscle glycogen, hematocrit, hemoglobin and Na^{+}

	Pre	**Post**	Δ
Body mass (kg)	78.6	72.7	-5.9
Muscle glycogen (mmol•kg^{-1} wet wt.)	152	48	-104
Hematocrit (%CV)	46	51	+5
Hemoglobin (g•dl^{-1})	15.6	17.3	+1.7
Na^{+} (mmol•L^{-1})	139	139	0

Based on the sub-maximal running pace, cycling power and expired gas data, regression equations were developed to estimate energy expenditure and energy substrate contribution (figure 1).

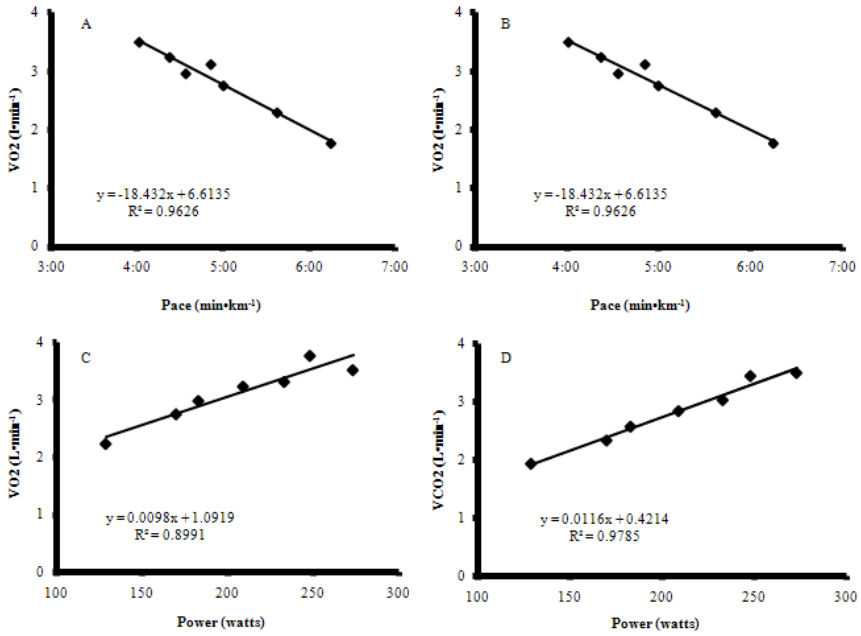

Figure 1. **A**. VO_2 regression equation for running. **B**. VCO_2 regression equation for running. **C**. VO_2 regression equation for cycling. **D**. VCO_2 regression equation for cycling.

Mode specific regression analysis closely estimated energy expenditure compared to the DLW method, while providing detailed oxidation rate and substrate use data. Water turnover, a dynamic measure of hydration flux, is a measure of the amount of water that is lost and replaced over the course of the event (table 1).

Table 2. Time, distance, and total energy expenditure during the DLW collection period.

	Total	**Swim**	**Bike**	**Run**	**Transitions**	**Non-race**
Time (min)	749.2	75.9	320	235.4	9.3	108.7
Distance (km)	225	3.9	179.2	41.9		
TEE (MJ)						
DLW	38.9					
equations	38.3	3.3	21.4	10.9	0.4	2.3
TEE (kcal)						
DLW	9290					
equations	9148	796	5120	2596	93	544
Energy Source (g)						
CHO	1341.6	128.5	925.0	288.1		
Fat	348.8	31.1	156.0	161.7		
rH_2O (L)	16.6					

Absolute power output and cycle intensity, as a percentage of peak VO_2 output, were similar during the half and full Ironman distance triathlons. Running pace and intensity, as a percentage of peak VO_2, were higher during the half compared to the full Ironman distance triathlon.

Mean power output for the cycle portion of the race was 210 watts (table 3), however, there was a steady decline in power production resulting in a decrease in energy expenditure during the cycle portion of the race (figure 2).

Table 3. Comparison of selected variables for the same athlete during a half and full Ironman triathlon.

	Half Ironman*	**Ironman**
%VO2 peak		
Cycle	68	64
Run	70	43
I**ntensity**		
Cycle (watts)	230	210
Run (min•km^{-1})	4.2	5.6
Glycogen use (% loss)	83	69
Carbohydrate oxidation (g)	1010	1213
Carbohydrate oxidation (g•min^{-1})	3.5	1.6

*Half Ironman was completed one year prior to the Ironman World Championship

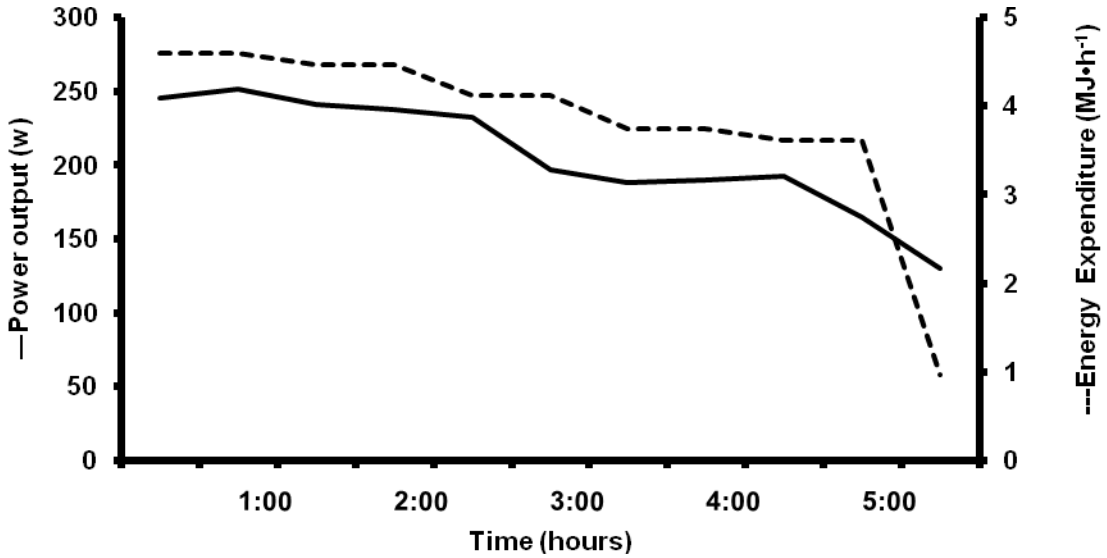

Figure 2. Mean power output and energy expenditure during the cycle portion of the race. Mean running pace was 5.6 min•km^{-1} with the running pace gradually increasing as the race progressed. Mean split times were 4.6, 5.7, and 6.1 min•km^{-1} for kilometers 0-8.3, 8.3-28.1, and 28.1-41.9, respectively.

Discussion:

The main findings of this study are that while doubly labeled water is the gold standard method for evaluating energy expenditure in a field setting the energy cost of activity can be closely predicted using specific regression equations based on the relationship of work to metabolic

demand. In addition to total energy expenditure the regression equations can also provide estimated energy substrate contributions.
While total carbohydrate oxidation is higher during the full Ironman, the rate of oxidation and the contribution of energy from muscle glycogenolysis are higher during the half Ironman triathlon. The carbohydrate oxidation rate (1.6 $g \bullet min^{-1}$) during this race was close to the maximal exogenous carbohydrate oxidation rate of 1.5 $g \bullet min^{-1}$. This demonstrates that at this intensity and with an aggressive feeding schedule carbohydrate oxidation could be nearly met by carbohydrate feeding. However, if sufficient carbohydrate supplementation is not maintained fatigue will occur and performance will suffer.

Fatigue is influenced by a myriad of factors during athletic events including hydration status, endogenous and exogenous energy availability, central nervous fatigue and elevated core temperature. During this Ironman World Championship two main factors, muscle glycogen and hydration, contributed to the reduction in performance particularly during the run portion. This race event demonstrates that while work intensity and carbohydrate oxidation are relatively low muscle glycogenolysis remains an important source of energy during activity. This athlete experienced a 69 percent reduction in muscle glycogen stores during the race. In addition to decreased muscle glycogen, this athlete also experienced severe dehydration. Performance can decline after only a one percent reduction of body mass due to hydration. This athlete lost 7.5 percent or 5.9 kg body mass. This amount of dehydration can cause both an imbalance in electrolyte concentration and a reduction in the body's ability to thermoregulate by sweating.

Conclusion:

Building regression equations based on an individuals' work output and gas data will provide an accurate estimate of carbohydrate vs. fat oxidation and total energy expenditure. Additionally, developing regression equations requires only minimal time and money investment while using double labeled water requires collection and analysis time, as well as, a substantial investment in the isotope heavy water and the analysis of the collected urine.

Field Based Study 9: Hydration Markers and Activity during Extreme Heat Stress and Ultra-Endurance Work: The Badwater Ultramarathon.

W. Hailes, D. Slivka, J. Cuddy and B. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Purpose:

The Badwater Ultramarathon is an extreme endurance foot race that requires the participants endure temperatures in excess of 40ºC (105ºF), over 3900 m (13000 ft) of ascent and 217 km (135 miles) of paved running in under 60 hours. Approximately 80 racers begin in three waves 6, 8 and 10 am for safety reasons and to minimize the impact on the national park. The race begins in Death Valley National Park, CA at the lowest point in North America 85 m (282 ft) below sea level. The first 41 miles are dominated by the excessive heat with only approximately 50 feet of elevation change. While there is less heat stress during the remaining 93 miles the racers must run over the Sierra foothills and finish by climbing to the Whitney Portal at 2533 m. This race provides a venue to study human performance during an event that combines extreme endurance and excessive heat. Due to ethical reasons it would not be feasible to subject research volunteers to the extremes of endurance and heat stress experienced during this event in a laboratory investigation. Therefore, using this race as a model it is possible to examine the unique adaptations and coping mechanisms adopted by individuals required to perform in similar environments. These individuals include; individuals racing similar endurance events, wildland firefighters working near a fire, warfighters on maneuver in hostile environments and others. The focus of this project was to investigate the relationships of activity intensity, core temperature and hydration status during extreme heat stress.

Method:

Subjects

Fourteen (male n = 11, female n = 3) racers volunteered to participate in this project during the 2008 Badwater Ultramarathon. Subjects included ultra-runners recruited from the Western United States. Prior to data collection, subjects provided written consent using an approved Institutional Review Board consent form (Protocol # 113-08).

Core Temperature
Subjects ingested a telemetric temperature sensing pill the evening before and the morning of the race. They also carried a small vital sense data logger that recorded the subjects' core temperature every minute during the first 41 miles of the race.

Body Mass
Body mass was measured (Belfour PS6600T, Saukville, WI, accuracy ± 100 g) immediately

prior to the race, after 41 miles and upon completion of the race.

Blood
Approximately 100µL of blood were collected pre race, at mile 41, and post race. The blood samples were immediately analyzed using the i-STAT (Abbot, East Windsor, NJ).

Activity Monitoring
Prior to beginning the race, all study participants were outfitted with an Actical activity monitor (MiniMitter, Bend, OR, USA) positioned on the non-dominate wrist according to manufacture guidelines. The Actical activity monitor utilizes an omni-directional accelerometer that measures motion in all planes and was set to record at one minute epochs. Once participants were outfitted with the activity monitors no adjustments were needed until removal after 41 miles of racing.

Results:

Blood samples demonstrated stable electrolyte concentration, except for a small but significant increase in potassium, during the first 41 mile of the race. By the end of the race sodium, chloride, and calcium all decreased relative to the pre race values while potassium returned to similar concentrations as pre race (table 1). Blood urea nitrogen and creatinine significantly increased during the first 41 miles of the race but did not increase further (table 1). Blood glucose increased significantly during the first 41 miles but returned to similar values as pre race by the end of the race (table 1). There was no change observed in body mass or urine specific gravity during the race.

Table 1. Physiological and environmental variables measured during the race

	Pre	**Mile 41**	**Post**
Sodium	**140 ± 2**	**139 ± 7**	**136 ± 3***
Potassium	**3.9 ± 0.3**	**4.3 ± 0.5***	**4.1 ± 0.3**
Chloride	**105 ± 2**	**105 ± 7**	**101 ± 3***
Ionized Calcium	**1.22 ± 0.04**	**1.20 ± 0.06**	**1.16 ± 0.04*†**
Glucose	**100 ± 15**	**127 ± 27***	**116 ± 25**
Urea Nitrogen	**14 ± 6**	**19 ± 7***	**24 ± 10***
Creatinine ($\mu mol \cdot L^{-1}$)	**0.9 ± 0.1**	**1.6 ± 0.6***	**1.3 ± 0.2***
Body Weight (kg)	**77.4 0 ± 9.8**	**77.3 ± 10.3**	**77.2 ± 9.8**
Urine Specific Gravity	**1.013 ± 0.009**	**1.020 ± 0.011**	**1.015 ± 0.011**
Temperature (ambient)	**35.2 ± 5.3**	**42.2 ± 1.0**	
Humidity (%)	**37.2 ± 9.7**	**18.3 ± 3.9**	

Values are mean ± SD. Values are $mmol \cdot L^{-1}$ unless noted. * Different than pre, $p<0.05$; †

different than mile 42, $p<0.05$.

Core temperature remained constant throughout the first 41 miles of the race (figure 1).

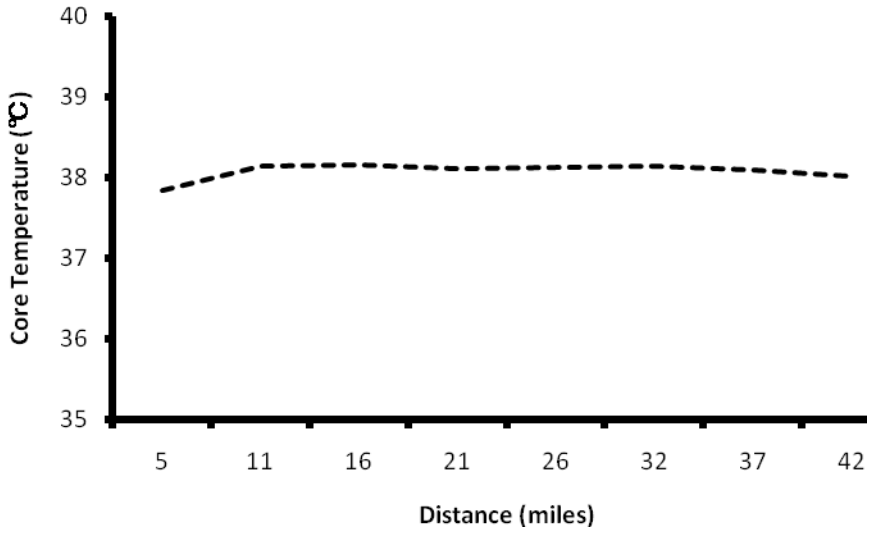

Figure 1. Core temperature (℃) during the first 42 miles of the race. (n=12)

There was a strong, significant correlation between mean activity count and pace for the first 17 miles of the race. There was not a significant correlation between activity count and pace from mile 18 to 41.

Table 2. The relationship between mean activity count and pace during the first 41 miles of the race

	Activity Count ($x10^3$) (counts • min^{-1})	Pace (min • $mile^{-1}$)	Pearson's r
Miles 0-17	6.2 ± 2.5	13 ± 1.9	-0.88**
Miles 18-41	5.4 ± 2.4	15 ± 2.0†	-0.15

Values are mean ± SD. **Significant correlation between activity count and pace, $p<0.01$; † Different than Miles 0-17, $p<0.01$.

Discussion:

Humans are the best distance runners on the planet in hot environments, out-performing dogs and horses. Our ability to thermoregulate is the main attribute that allows humans to run longer than any other animal. This ability is highlighted in an event such as the Badwater Ultramarathon. Death Valley consistently records the highest temperatures in the United States. The mean high temperature in July, the month of the race, is 46ºC (115ºF) with a record high of 57 ºC (134ºF). While this area is very hot it is also very dry, which allows dissipation of heat by sweating. Sweating is the most effective method, available to humans, of thermoregulation in

hot and dry environments. During the hottest portion of the race the participants were able to maintain core temperature, and blood electrolyte concentrations similar to pre race concentrations without any change in body mass or urine specific gravity. The maintenance of core temperature demonstrates the effectiveness of the human cooling system. While the maintenance of electrolyte concentration, body mass and urine specific gravity demonstrates the skill of runners. These runners demonstrate that with proper nutrition and hydration it is possible to work during extreme heat stress and maintain homeostasis.

These runners maintained homeostasis under extreme heat stress, however, electrolyte concentration and blood glucose at the end of the race were reduced. Our hypothesis is that with the reduction in heat stress as night approached and after many hours of racing, participants were less focused on hydration and nutrition. Over many miles the reduced intake led to the observed shift away from homeostasis.

The runners were able to maintain physiological homeostasis during the first 41 miles of the race, however, they were not able to maintain pace. The mean pace increased approximately 2 minutes per mile during miles 18 to 41 compared to the first 17 miles. This decreased speed was anticipated and our hope was to determine if individuals adjusted speed to maintain a stable core temperature. Because wrist mounted GPS units are incapable of monitoring for this duration we decided to use activity monitors as an indicator of pace. Previous unpublished data from our laboratory shows a significant correlation between pace and activity during shorter running events of constant pace. However, during this event there is a breakdown in the relationship between running speed and activity. There is a strong, significant correlation between pace and activity during the first 17 miles of this race, however, there is no significant correlation between the pace and activity during the subsequent 24 miles. Terrain during the first 41 miles of this race was very constant therefore we assume the varied modes of travel from slow walking to running affected the relationship between speed and activity.

Conclusions:

The current data demonstrate that individuals can maintain core temperature and proper hydration during endurance activities in high heat stress environments, if proper nutrition and fluids are available. Unfortunately, while homeostasis was maintained during activity in heat stress a reduction in work output was observed. Further research is needed to determine the exact cause of the reduced work.

Field Based Study 10: Total Energy Expenditure, Body Water Turnover, and Hydration Status during the Western States 100: A Model for Extended Military Operations.

W. Hailes, D. Slivka, J. Cuddy and B. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

Many individuals are performing intense work in hostile environments for extended periods of time; some examples include wildland firefighters, elite warfighters, and ultra-endurance runners. It is important for these individuals to understand the response of the human physiological system during extended work scenarios so that proper training, nutrition, and hydration strategies can be implemented. Additionally, by understanding the responses to extended work, maintenance habits can be modified during an extended work scenario to increase the individuals' performance and therefore safety. Participants in the Western States 100 ultra-endurance race provide a model of endurance performance that includes extended work in hot and cool environmental stress, at moderately high altitude and at an intense pace commiserate with a race event. The purpose of this study is to monitor blood, urine and isotopic markers of hydration status, renal function and total energy expenditure of individuals completing the Western States 100 Ultra-endurance race. The results of this study will provide energy requirement and hydration stress data that can help endurance runners, elite warfighters or any individual involved in extended work operations optimize performance.

Methods:

Ten subjects recruited from the Western United States (5 male, 5 female, 42.8±8.1yrs, and 70.0±11.6 kg) completed the 100 mile run within the 30 hour time limit. These subjects responded to an advertisement for volunteer study participants on the official race website. The event is a 100 mile trail run completed as quickly as possible. The course begins in Squaw Valley Ski resort; California ascends approximately 5455m and descends approximately 7000 m while crossing the Sierra Mountains ending in Auburn, California. Prior to data collection, the protocol was approved by the institutional review board (Protocol #57-07).

Water Turnover and TEE

A dose of water that contains a higher than normal amount of a stable isotope for hydrogen (^{2}H) and oxygen (^{18}O) was administered orally after the collection of a background urine sample. After consumption of the original dose mixture, the dose vial was rinsed three times with tap water to ensure complete isotopic delivery (approximately 20 mL per rinse). Subjects were required to refrain from the consumption of food or additional water until first void urine samples the following morning. Second void urine samples were then collected for analysis. After the first void, a nude body mass was obtained (Belfour PS6600T, Saukville, WI, accuracy

± 100 g). All overnight voids were collected to correct the measure of initial Total Body Water (TBW). TBW was calculated from the change in hydrogen isotope enrichment (background vs. the second void urine). Urine samples were also collected at specific locations along the race course so that total energy expenditure could be calculated by measuring the speed of oxygen isotope elimination. Urine was collected immediately pre race, at mile 55.7, and immediately post race. Body mass was measured pre race, at mile 66.9, and immediately post race.

Blood
Blood was collected pre race, at mile 66.9, and immediately post race. The blood samples were immediately analyzed using the i-STAT (Abbot, East Windsor, NJ).

Results:

Males were heavier than females and had significantly higher total body water, but did not experience higher water turnover or energy expenditure during the duration of the race. Females did have lower energy expenditure per hour during the race (Table 1).

Table 1. Sex comparison of size, energy expenditure and water flux during and ultra-endurance run

	Male (n=5)	**Female** (n=5)
Body Mass (kg)	**78.4 ± 8.0**	**61.5 ± 7.7***
Total Body Water (L)	**49.9 ± 5.5**	**37.7 ± 4.1***
Water Turnover (L•race^{-1})	**19.8 ± 2.3**	**16.1 ± 2.8**
Water Turnover (mL•kg^{-1}•race^{-1})	**254.1 ± 37.1**	**259.3 ± 48.8**
Energy Expenditure (MJ•race^{-1})	**72.4 ± 8.8**	**60.2 ± 13.0**
Energy Expenditure (kcal•race^{-1})	**17,319.9 ± 2103.2**	**14,400.9 ± 3110.0**
Energy Expenditure (kcal•hour^{-1})	**657.0 ± 59.5**	**527.7 ± 69.5***
Energy Expenditure (kcal•kg^{-1})	**221.3 ± 23.4**	**228.0 ± 23.2**

Values are mean ± SD. * Different than Male, $p<0.05$

There were fluctuations in potassium, chloride and ionized calcium during the race event while sodium did not change significantly. At the midpoint blood glucose was increased over pre race but returned toward pre race values by the end of the race. Urea nitrogen and creatinine both increased throughout the race (Table 2). Body mass decreased during the first portion of the event and returned toward pre race values by the end. Urine specific gravity increased during the initial portion of the race and maintained at the elevated level until the end (Table 2).

Table 2. Blood, body mass and urine measures during an ultra-endurance run (n=10)

Physiological Measure ($mmol \cdot L^{-1}$ unless noted)	Pre	Mid	Post
Sodium	**141 ± 2**	**140 ± 2**	**138 ± 4**
Potassium	**4.4 ± 0.3**	**4.2 ± 0.4**	**3.8 ± 0.4***
Chloride	**104 ± 1**	**106 ± 2***	**103 ± 4†**
Ionized Calcium	**1.3 ± 0.0**	**1.2 ± 0.1***	**1.2 ± 0.1***
Glucose	**5.0 ± 0.4**	**7.3 ± 1.2***	**6.4 ± 1.6†**
Urea Nitrogen	**6.2 ± 2.0**	**9.7 ± 2.1***	**10.4 ± 4.3***
Creatinine ($\mu mol \cdot L^{-1}$)	**94 ± 15**	**126 ± 31***	**122 ± 29***
Hematocrit (%PCV)	**44.1 ± 1.8**	**42.1 ± 3.5**	**40.9 ± 3.5**
Hemoglobin	**9.3 ± 0.4**	**8.9 ± 0.7**	**8.6 ± 0.8**
Body Mass (kg)	**157 ± 26**	**154 ± 24***	**155 ± 24**
Urine Specific Gravity	**1.015 ± 0.008**	**1.029 ± 0.005***	**1.023 ± 0.008***

Values are mean ± SD. * Different than pre, $p<0.05$; † different than mid, $p<0.05$.

Discussion and Conclusion:

The main findings of this study are that during an extended race scenario participants experience large amounts of water flux, signs of dehydration and renal dysfunction while maintaining sufficient energy and sodium supplementation.

The runners completed 100 miles in an average of 26.8 ± 3.1 hours of racing while enduring diverse climatic conditions and demanding terrain. The runners in this study withstood an enormous energy (66.3 ± 12.3MJ) and water (17.9 ± 3.1L) flux during this event. During the run the subjects were able to maintain a normal blood sodium concentration and an increased blood glucose concentration compared to pre race, demonstrating an adequate consumption of both sodium and carbohydrates. Potassium gradually fell from pre race to post race indicating that adequate consumption was not being maintained, which can lead to muscle and nerve dysfunction.

Markers of dehydration such as an increased urine specific gravity and a decrease in body mass after 66.9 miles of racing were also apparent. Additionally, blood urea nitrogen and creatinine were elevated indicating a reduced glomerular filtration rate caused by a combination of increased protein catabolism and dehydration. This reduced renal function could have affected

the rate of urine elimination, promoting water retention and the weight gain seen between mile 66.9 and the end of the race. Although urine volume was not monitored, we hypothesize that the weight gain was primarily due to the reduced thermal stress as the runners entered the night portion of the race, not due to the reduced renal function. This hypothesis is based on the relatively minor and acute disruption of renal function caused by the race event and by the continued elimination of potassium and the plateau of urine specific gravity at an elevated value for the second portion of the race.

Based on this study total energy intake and hydration are the most limiting factor for participants in extended work scenarios. Maintaining energy consumption equal to energy expenditure is impractical in this relatively short period of work. However, performance may be best maintained by ingesting small, frequent amounts of supplemental carbohydrate sources during an event of this duration. These athletes were able to maintain blood sodium concentrations compared to their pre-race concentrations, however, potassium and calcium concentration fell. This could be remedied by increased focus on more balanced electrolyte supplementation. The water turnover or the amount of water that is lost and replaced, of 17.9 ± 3.1 L demonstrates that the 17.9 L of water ingested was not sufficient to maintain hydration. Ideally, ultra-endurance athletes would consume more water resulting in a more stable body mass, a lower specific gravity and increased glomerular filtration rate helping the body clear excessive blood urea nitrogen and creatinine.

Field Based Study 11: Applicability of the Actical to measure intensity and duration of activity during high altitude expeditions.

W. Hailes and B. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

Ambitious high altitude research expeditions, such as the "Silver Hut" expedition of 1962 have aided our understanding of adaptation to hypobaric hypoxia and performance in high altitude mountainous regions. As researchers expand the scope of high altitude, field based scientific investigation it will become increasingly important to measures the work intensity of the research participants. Accelerometer-based studies have evaluated the physical activity of many populations in various environments.

The ability to measure activity intensity, recorded as counts per unit time, along with the small size ($2.8x2.7x1.0cm^3$), water resistance, and data recording potential (up to 45 days) makes activity monitors very attractive for use in remote mountainous environments. Value calibration of the activity monitor is needed to translate the raw counts into a more meaningful measure such as physiological intensity or energy expenditure. Calibration has occurred for fast and slow locomotion, activities of daily life, and wildland firefighter activities but similar calibrations have not occurred at high altitudes or during mountaineering activities. Without such calibration the raw data counts cannot provide metabolic information but can still provide the intensity range and time course of activity.

Previous scientific investigations in high altitude mountainous environments have examined important physiological phenomena such as acute mountain sickness (AMS), pulmonary and cerebral edema, energy expenditure, and cold exposure. However, to the authors knowledge intensity of exercise and activity patterns during multiple day ascents of large mountains have not been investigated. The purpose of the present investigation was to describe the intensity and duration of activity during a climbing expedition to 6194m using activity monitors.

Methods:

Seven healthy males recruited from the western part of the United States (40.6±11.5 yrs, 179.6 ± 3.5 cm, 77.3 ± 8.8 kg) with previous climbing experience served as participants in the present investigation. The study was approved by the Intstitutional Review Board at The University of Montana (Missoula, MT, USA; Protocol #69-07), and all subjects provided written informed consent prior to data collection. Participants were part of a commercially guided climbing group consisting of both guides and climbers attempting to summit Denali (Alaska, USA) via the West Buttress route during May 2007. All participants moved as a group and performed similar activities during the study except for the following: on day eight it was required that two guides

separate from the group resting at 3394m, descend to 2121m and return to the group later that day, traveling a total of 29.5km in approximately 12 hours; on day 15-four individuals, the summit team (ST), traveled from 4300m to high camp at 5212m while three individuals, the early descent team (ED), and began their two day descent from 4300m. On day 18, the ST reached the summit and began their two day descent on day 19. Figure 1 shows a detailed profile of the climb.

Prior to beginning the climb, all study participants were outfitted with an Actical activity monitor (MiniMitter, Bend, OR, USA) positioned on the non-dominate wrist according to manufacture guidelines. The Actical activity monitor utilizes an omni-directional accelerometer that measures motion in all planes and was set to record at one minute epochs. The activity monitors recorded data for the duration of the study enduring the cold and high altitude. Once participants were outfitted with the activity monitors no adjustments were needed for the duration of the investigation. A global positioning system unit (eTrex Vista Cx, Garmin Ltd., Olathe, KS, USA) was used to record distance traveled and altitude. Pre and post climb body mass was collected using an electronic scale (Newline, Hicksville, NY) to the closest 0.09kg.

Data analysis

Mean activity counts were 1) partitioned into 100 count•min^{-1} sections to show activity intensity distribution (fig 2), 2) separated by day to show average counts•day^{-1} (fig 1), and 3) divided into travel and rest days when all participants were together (days 1-9 and 11-16). Activity intensity (counts•min^{-1}) was divided and analyzed based on 1) a net increase in altitude (3 days), 2) a net decrease in altitude (2 days), 3) camp activities (15 days), and 4) activity between the hours of 10:30 pm and 6:30 am (14 nights).

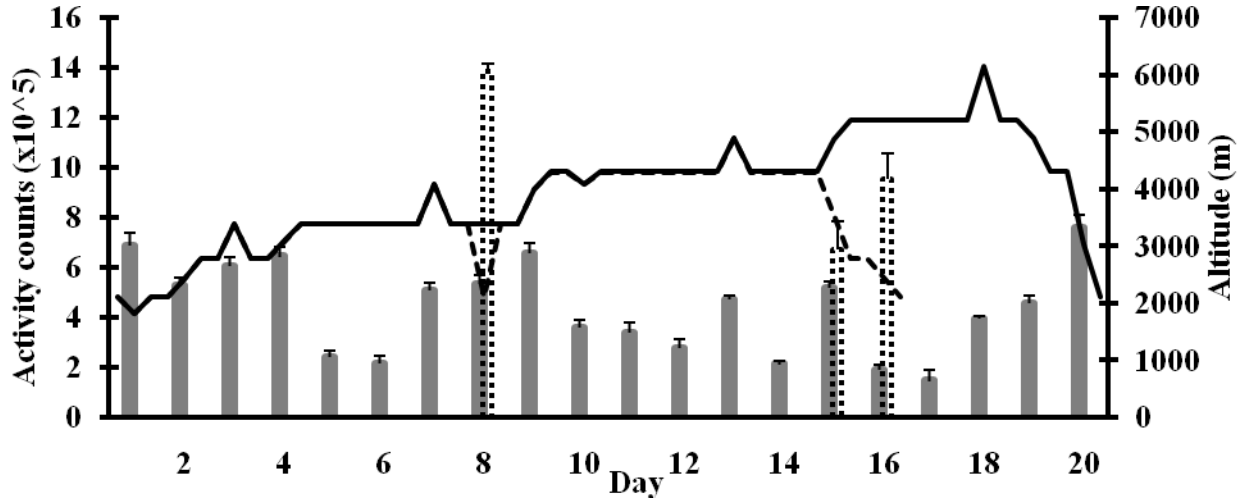

Figure 1. Total activity counts (x10^5) (bars) and ascent profile (lines) per day. Day 8, guides (dashed line and bar) traveled for supplies while climbers (solid line and bar) rested in camp. Day 15, ED group (dashed line and bar) descended while ST group (solid line and bar) continued climbing.

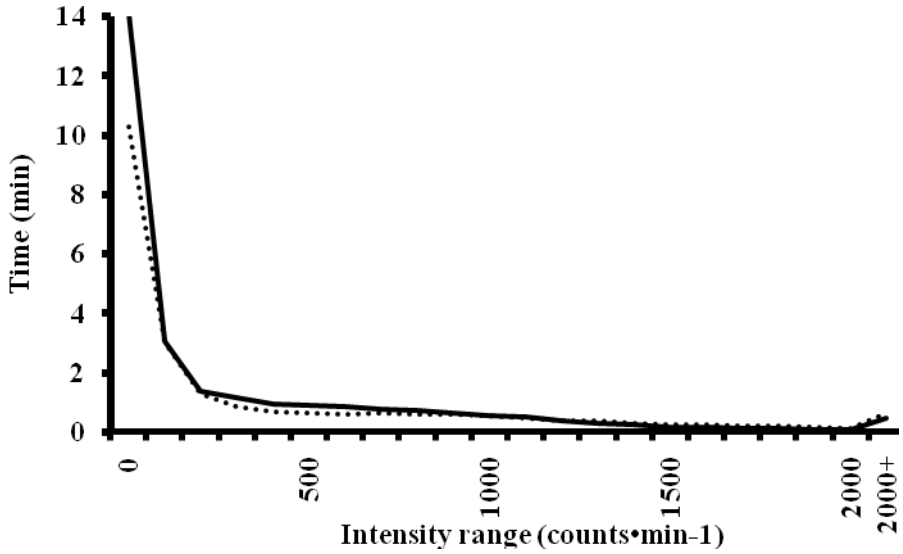

Figure 2. Amount of time spent in various intensity ranges. —Summit team, ---Early descent

The participants were divided 1) into a summit team (ST, n=4) and an early descent team (ED, n=3) and 2) by their skill level as either guides (n=2) or climbers (n=5). Data were analyzed using independent t-tests and the level of significance was set at an experimental alpha level of $p<0.05$. Individuals' complete climb activity data were segmented by day and correlated with daily distance traveled. Mean pre and post body weights were compared using a dependant t-test. The level of significance was set at an experimental alpha level of $p<0.05$. Data are presented as mean ± standard deviation (SD).

Results:

Days 1-19

When evaluating the total climb, the total activity counts of the guides were significantly higher than the climbers ($9.9x10^6 \pm 3.4x10^5$ and $7.7x10^6 \pm 9.8x10^5$, respectively). There was no difference in total activity counts between the ST and ED groups ($8.2x10^6 \pm 1.6x10^6$ and $6.5x10^6 \pm 1.1x10^6$ counts, respectively). However, the ST spent more time at an activity level of 0 counts•min^{-1} than the ED group (234.9±23.7 vs. 171.3±23.8 hours, respectively).

Days 1-9 and 11-16

There was no difference in total activity counts between the guides ($8.6x10^6 \pm 8.8x10^5$) and the climbers ($6.4x10^6 \pm 1.4x10^6$) or between the ST ($6.1x10^6 \pm 1.5x10^6$) and ED ($8.2x10^6 \pm 1.1x10^6$) while all participants were together (days 1-9 and 11-16). During the same 15 days there was a difference in total activity counts between the travel and rest days ($5.6x10^5 \pm 1.1x10^5$ and $2.7x10^5 \pm 5.3\ x10^4$, respectively).

Activity intensity

There was no difference in activity intensity between the guides and climbers during any portion

of the climb. The ED group displayed increased activity during overnight periods and during the descent compared to the ST group (Table 1).

Table 1. Activity intensity (counts•min^{-1}) (±SD) of the total (n=7) group and the group divided by climb schedule and experience during four portions of the climb. * p<0.05, compared to ED.

	Total	ED	ST	Guide	Climber
Increased altitude	725±147	792±100	674±160	755±139	712±154
Decreased altitude	1011±225	1152±100	905±239*	1110±201	971±231
Camp	352±71	384±60	329±78	409±8	329±74
Overnight	16±13	19±17	13±9*	14±15	16±13

There was a correlation (r^2=0.62, p<0.0001) between distance traveled and activity counts (Fig 3).

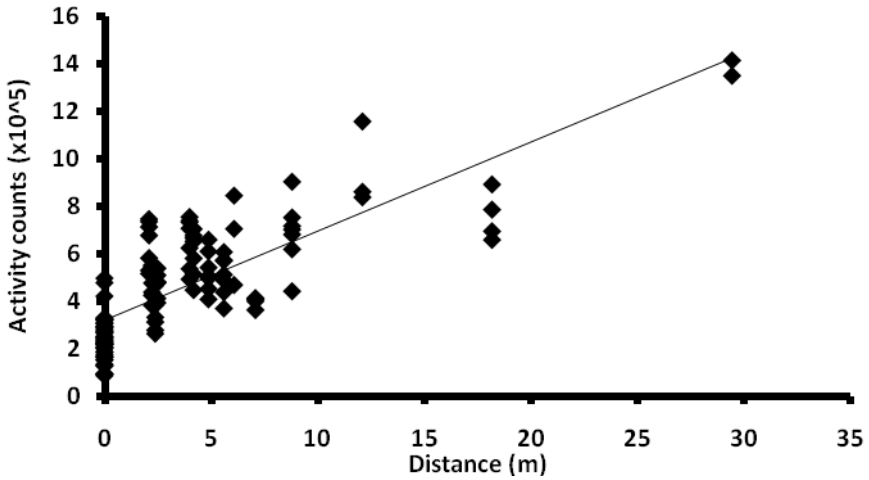

Figure 3. Activity counts and distance traveled per day.

Examples of mean activity data for typical travel and rest days and for the day that participants climbed to the summit are presented in Fig 4. Body mass decreased from 78.2±8.7kg pre to 76.6±8.9kg post climb.

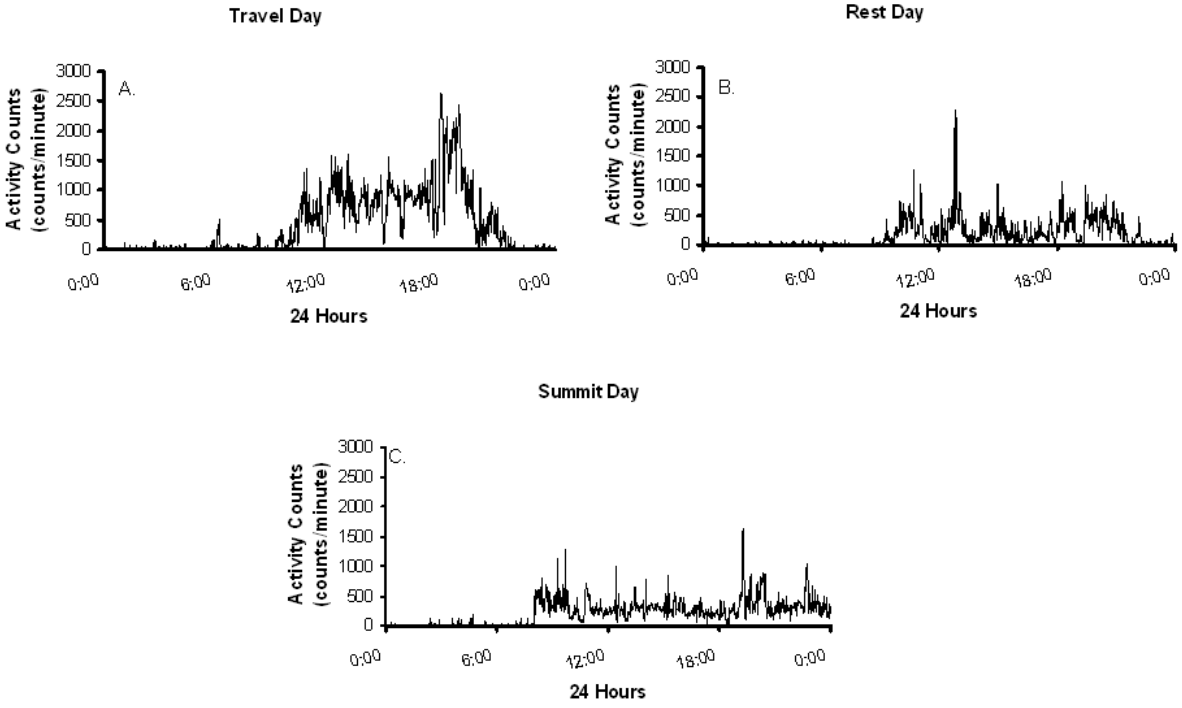

Figure 4. A. Mean activity data for all participants on a typical travel day. n=7 B. Mean activity data for all participants during a typical rest day, no travel. n=7 C. Mean activity data on the day that participants climbed to the summit (n=4)

Discussion:

Objectively quantifying the physical demands of mountaineering is a difficult task. While determining the total energy expenditure via the doubly labeled water method during expedition climbing provides a measure of whole body work output it cannot isolate periods of higher intensity or the time spent at various levels of exertion. Previous research has evaluated the total energy expenditure of climbing to and staying at high altitudes, but to our knowledge this is the first attempt to use activity monitors to describe activity during a remote high altitude expedition.

The data demonstrate the expected result that participants were more active on days requiring travel from one location to another compared to days spent in camp resting. When the group was divided by experience, the guides were more active than the climbers over the duration of the climb. The increase in guide activity was due to their additional travel on day 10 while the climbers rested. When comparing guides and climbers on days where the entire group was together there were no differences in activity, thus indicating that the added responsibility for daily work activities, like cooking and collecting snow for water did not make a large

contribution to the higher total activity counts. Additionally, the correlation between daily activity counts and distance traveled (Fig. 3) shows that there is a strong relationship between locomotion and activity in this environment.

Total activity counts were not different between the ST and ED groups even though the ST group spent four additional days on the mountain, traveling 23km farther and ascending 1994 additional meters. This can be explained by the combination of large individual variation in activity counts and that the two days spent resting at the high camp (5200m) contributed relatively minor increases in total activity counts as demonstrated by the additional 63.6 hours spent at an activity intensity of 0 counts•min^{-1} for the ST. However, the ED did show increased activity intensity during the overnight and descent portions of the climb compared to the ST group. The reduced intensity of downhill walking by the ST group could be explained by the difference in terrain descending from 5212 to 4300m, terrain the ED group did not descend. This portion of the mountain requires the use of fixed safety lines and more careful route planning, resulting in decreased activity intensity. The increased activity intensity of the ED group during the overnight hours could result in these individuals not getting the amount or quality of sleep as the ST group; however, it is beyond the scope of this investigation to determine sleep quality.

One important consideration when evaluating activity intensity using accelerometer-based activity monitors is terrain. It is physiologically more demanding to walk uphill than downhill, however, activity monitors record higher rates of activity during downhill locomotion (Table 1). Therefore measuring course profile or slope is important to accurately assess activity intensity in mountainous environments.

Roach and associates demonstrated that exercise exacerbates the symptoms of AMS at simulated high altitude. While investigators are able to control the participants intensity and duration of exercise in the laboratory, this control is not possible when investigating AMS during mountain expeditions. Therefore, field investigations into altitude sickness should include measures of exercise intensity and duration. The current data demonstrates that activity monitors can provide a useful tool for the measurement of exercise intensity and duration in rugged field trials such as mountain expeditions.

Previous research has shown that high intensity exercise negatively alters the blood-gas barrier in trained athletes due to increased capillary pressures, and during acute exposure to high altitude, increased capillary pressure has been shown to be the initial cause of high altitude pulmonary edema. However, Bircher and associates determined that physical fitness and mean heart rate during ascent were minimally important factors in the development of AMS and pulmonary edema (1994). Mean heart rate does not accurately describe the range or duration of exercise intensities. Therefore, future research is needed to determine the impact of exercise intensity on the susceptibility of altitude related illnesses. The use of activity monitoring may provide a useful tool in the identification of activity patterns that increase the occurrence or severity of AMS, pulmonary or cerebral edema.

Activity monitors could also become important tools for climb management purposes. The coordination of arterial saturation, sleep quality, AMS symptomlogy and stress accumulation (based on activity counts) could help climbers or medical support optimize climber rest: ascent

schedules to decrease AMS symptoms and increase safety.

Conclusion:

While further research is needed to broaden the use of activity monitors to estimate energy expenditure or provide a clinical/safety management tool while climbing, this study demonstrates that activity monitors can be used to describe activity patterns of individuals performing in remote high altitude environments.

Field Based Study 12: Core temperature during consecutive running races.

J. Cuddy and B. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

The measure of core body temperature has not been fully evaluated during competitive exercise settings outside of the laboratory. The purpose of this study was to determine the variations in core body temperature during competitive running. The study took part during the 2008 YMCA Riverbank Run Trifecta, which consists of three back-to-back races: 10K, 5K, and 1 Mile. Participants included 13 males from the local running community.

Methods:

Prior to data collection, the protocol was approved by the Institutional Review Board (93-08). Subjects included highly trained male runners from the Missoula Community.

Prior to the race, subjects completed several laboratory tests: skinfold and hydrostatic weighing body fat assessment, and a maximal exercise treadmill test to exhaustion. The anthropometric measures (age, height, weight, % body fat, fat free mass, and fat mass) are used to measure the absolute and relative variability in size and shape of the participant pool. The maximal treadmill test indicates one's ability to use oxygen at the cellular level to complete mechanical work, and can be used to estimate race performance and fitness changes over time.

On race day, subjects ingested a core temperature sensor (size of a vitamin pill, see picture below) and were equipped with a waist pack containing a unit to record data transmitted from the sensor. Additionally, several participants were equipped with a patch sensor that records environmental temperatures. Participants were also outfitted with a wrist worn GPS unit to monitor running speed, as well as an ActiCal® activity monitor to evaluate subtle changes in speed and/or running efficiency. Following the race, researchers downloaded data onto a computer (in case you are wondering, we allowed participants to keep their core temperature sensors).

Results:

Table 1. Descriptive Data (N=13). Values are mean ± standard deviation.

Age (years)	30 ± 6
Height (cm)	182 ± 3
Weight (pounds)	75 ± 6
% Fat Underwater	14 ± 5
% Fat Skinfold	10 ± 5
Fat Free Mass (pounds)	65 ± 4
Fat Mass (pounds)	10 ± 4
Max O^2 Uptake ($L \cdot min^{-1}$)	4.9 ± 0.6
Max O^2 Uptake ($ml \cdot kg \cdot min^{-1}$)	65 ± 9

Table 2. Race Data (N=13). Values are mean ± standard deviation.

	Time (min)	Pace (min/mile)	Speed (mph)	Race VO_2 ($L \cdot min^{-1}$)	Race VO_2 ($ml \cdot kg \cdot min^{-1}$)	Race Pace % Max VO_2
10K	37:28 ± 4:22	6:03 ± 0:42	10.1 ± 1.2	4.3 ± 0.6	57 ± 9	88 ± 3
5K	17:55 ± 2:04	5:47 ± 0:40	10.5 ± 1.2	4.5 ± 0.6	59 ± 9	92 ± 3
1 Mile	5:29 ± 0:28	5:29 ± 0:28	11.0 ± 1.0	4.7 ± 0.5	62 ± 8	96 ± 4

Table 3. Core Temperature Data (N=10). Values are mean ± standard deviation.

	Mean Temp (°C)	Mean Max Temp (°C)	Mean Max Temp (°C)
10K	38.6 ± 0.5	39.1 ± 0.4	37.6 ± 0.6
5K	37.8 ± 0.6	38.6 ± 0.4	36.9 ± 1.0
1 Mile	37.6 ± 0.8	37.8 ± 0.7	37.3 ± 0.9

** 98.6°F = 37°C; 40°C is a laboratory cutoff for exercise*

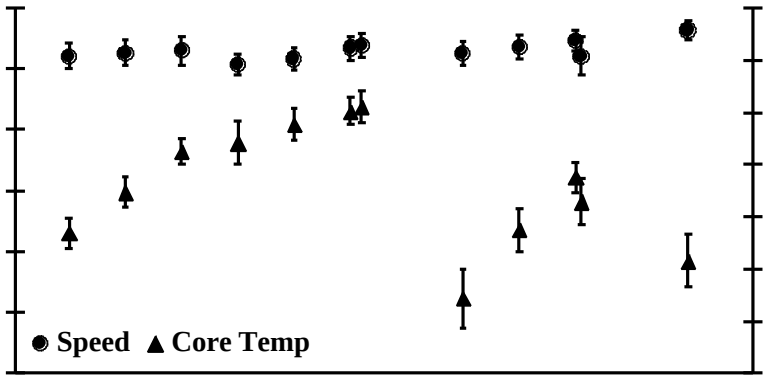

Figure 1. Speed and Temperature during 3 consecutive races.

Discussion:

While this study was primarily a pilot study for future investigation with core temperature monitoring during running, it was interesting that core temperature in the 5K and 1 Mile race did not reach the core temperature achieved in the 10K. Originally we hypothesized that core temperature would remain elevated between races (possibly a slight drop), but temperature returned to values similar to baseline prior to the start of both the 5K and 1 Mile race. Thus, participants were "cooling down" in between races, and then the duration of a 5K and/or 1 Mile race was too short to elevate temperature to the levels of the 10K. This implies that during race conditions with ideal running weather (sunny, 54-63°F), it takes considerable time to elevate core body temperature. So, what do the results of this study mean for recreational runners? Since warmed up muscles perform better, an adequate warm up prior to shorter races in moderate to cold temperatures is critical. The warm up should not only include a period of easy jogging, but should include some moderate to high intensity bouts to elevate muscle temperature. Additionally, the warm up should be done close to race time so the body does not have time to cool down.

Field Based Study 13: Adaptations to periods of intensified training; Implications and effectiveness of high volume training camps.

D. Slivka, W. Hailes, J. Cuddy and B. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

Many endurance athletes enter a period of high volume training as part of an early season "base" training or in preparation for a priority sporting event. The purpose of these intensified periods of training is to gain fitness quickly. It is unclear if these periods of training lead to positive training adaptations or if the high volume nature of the training does not allow for adequate recovery and thus an over-training stimulus. Additionally, the time course of adaptations during periods of intensified training is unknown. The purpose of this study is to implement a period of intensified training in physically fit individuals to assess the time course and magnitude of physiological adaptations. These results will elucidate the implications and effectiveness of periods of intensified training.

The results of this data may be applicable for not just the endurance athlete, but also any individual looking to improve fitness in a short period of time. This model may be used as a military pre-deployment strategy in order to optimize training strategies for optimal fitness when time may be a limiting factor.

Methods:

Subjects

Ten (n = 10) male participants were recruited from the local triathlon and cycling community. These individuals were recruited due to their success in local endurance racing events and were judged by the investigators of having a realistic chance of completing the protocols involved with the current investigation. The protocol was approved by the Institutional Review Board (Protocol #243-06).

Training, living conditions, and stresses

The period of intensified training was organized as a 21 day out and back bicycle tour. Participants cycled at a self-selected work rate for the bulk of the training (except the 1 hr performance trial, see overtraining report). A hot breakfast and dinner were provided from a catering service that specialized in bicycling tours. During time spent cycling, participants were provided with commercially available sports food bars and drinks. Nights were spent at campgrounds in tents. By providing these accommodations daily stresses, outside of the daily cycling exercise, were minimized.

Biopsies
Biopsies were taken from the *vastus lateralis* muscle of using a 4-5 mm Bergstrom percutaneous muscle biopsy needle (Bergstrom 1962). Each successive biopsy on the same leg was obtained from a separate incision 2 cm proximal to the previous biopsy. After any excess blood, connective tissue, or fat were removed the tissue samples were immersed in liquid nitrogen and stored at -80°C for later analysis. Biopsies were obtained pre and post exercise on days 1, 11, and 21 for the analysis of muscle glycogen and metabolic enzymes (see below).

Metabolic Enzymes
Muscle (~10 mg) was mechanically homogenized in CelLytic MT mammalian Tissue Lysis/Extraction Reagent (Sigma #C3228). For B-HAD, 20 ul of homogenate was added to 970 ul of a reaction solution (6 ml 1mM EDTA, 4 ml 1M Imidazole (Sigma #68268), 3.5 ml 1mM NADH (Sigma #N4505-100mg), and 86.5 ml distilled water) and 10 ul of 5 mM Acetylacetyl Co-A (Sigma #A1625). The elimination of NADH was then followed for 90 seconds at 340 nm. Enzyme activity was calculated with the extinction co-efficient for NADH (6.22). For Citrate Synthase, the same muscle homogenate was used. Citrate synthase was assayed using a citrate synthase assay kit (Sigma #CS0720) according to the manufacture specifications. All measurements were taken at 25°C.

Glycogen
Muscle glycogen was analyzed using an enzymatic spectophotometric method. Samples were weighed upon removal from an -80°C freezer and placed in 0.5 ml, 2N HCl solution. The sample solutions were weighed, incubated for two hours at 100°C in an oven, then re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5ml of 0.67 NaOH was added. A volume of this muscle extract (20μl) was added to 1 ml of Infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd.) and read on a spectrophotometer at 340nm. Muscle glycogen was then calculated using the extinction co-efficient of NADH. Muscle glycogen concentrations are expressed in $mmol \cdot kg^{-1}$ wet weight of muscle tissue.

Body Composition
Body density was determined using hydrodensitometry and corrected for estimated residual lung volume. Net underwater weights were recorded using a digital scale (Exertech, Dresbach, MN). Body density was then converted to body composition using the Siri equation.

Water Turnover
A dose of water that contains a higher than normal amount of a stable isotope for hydrogen (^2H) was administered orally (approximately 9-10 grams). Study participants were provided with an oral dose of ^2H_2O (99% ape, approximately 9 g mixed in approximately 35 mL of tap water, Cambridge Isotope Laboratories, Andover, MA) after the collection of a background urine sample. After consumption of the original dose mixture, the dose vial was rinsed three times with tap water to ensure complete isotopic delivery (approximately 20 mL per rinse). Subjects were required to refrain from the consumption of food or additional water until first void urine samples the following morning. Second void urine samples will then be obtained. After the first void, a nude body mass will obtained (Belfour PS6600T, Saukville, WI, accuracy ± 100 g). All

overnight voids will be collected to correct the measure of initial Total Body Water (TBW). TBW was calculated from the change in isotopic enrichment (background vs. the second void urine). Each urine sample was then mixed with ca. 200 mg of dry carbon black and filtered through a 0.22-µm filter to remove particulate materials and much of the organic material. Two 1-mL aliquots of each specimen were then placed in 2-mL septum sealed, glass vials. Deuterium analysis is performed by reducing 0.8 µL of cleaned fluid over chromium at 850°C which produces pure H_2 gas that is introduced to a Finnigan MAT Delta Plus isotope ratio mass spectrometer. Deuterium abundance is measured against a working standard using a standard dual inlet, Faraday Cup, differential gas isotope ratio procedure. Enriched and depleted controls are analyzed at the start and end of each batch, and these secondary standards used to calculate the "per mille" abundance versus Standard Mean Ocean water for each urine sample. All analyses were performed in duplicate, and all specimens from the same participant analyzed during the same batch. Results were corrected for any memory from the previous chromium reduction process. If duplicates differed by more than 5 per mil, duplicate analyses were repeated. Isotope dilution space was calculated as described by Cole and Coward. TBW was calculated by averaging the deuterium dilution space/1.041.

Exercise capacity

Maximum oxygen consumption (VO_2max) and workload associated with VO_2max was measured for each subject using a graded exercise protocol (starting at 95 watts and increasing 35 watts every three minutes) on an electronically braked cycle ergometer trainer (Computrainer, RacerMate Inc., Seattle, WA). Participants rode to volitional fatigue. Maximum workload was calculated as the highest completed stage (in watts) + the proportion of time multiplied by the 35 watt stage increment. Expired gases were collected during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and recorded at 15-second intervals. During each stage, the average expired gas values for the last 1 minute was recorded for the calculation of Respiratory Exchange Rate (RER) and sub-maximal substrate oxidation

Statistics

Differences in training parameters over training days were compared using one-way repeated-measures ANOVAs. Glycogen was compared over training days and by time of day (pre vs. post acute exercise) using two-way repeated-measures ANOVA's. In the event of a significant F ratio the false detection rate method (Benjamini and Hochberg 1995) was applied (R Development Core Team 2007) to locate differences and correct for multiple comparisons. All ANOVAs were performed using SPSS for windows Version 9 (Chicago, IL). A probability of type I error less than 5% was considered significant ($p<0.05$). All data are reported as means ± SE.

Results:

Metabolic Enzymes

Citrate synthase activity increased from day 11 (31.45 ± 0.97 umol/min/g) to Day 21 (34.84 ± 1.05 umol/min/g; $p<0.05$), but was no different than Day 1 (31.69 ± 1.51 umol/min/g). See figure 1. B-HAD activity did not change over the course of the 21 days of intensified training (day 1, 5.59 ± 0.28; day 11, 5.04 ± 0.26; day 21, 5.36 ± 0.28 mm/kg/min). See figure 2.

Muscle Glycogen
Muscle glycogen deceased during exercise on Day 1, Day 11, and Day 21. Muscle glycogen was higher during Day 11 and Day 21 than it was during Day 1. However, there was no difference between Day 11 and Day 21. There was no difference in glycogen utilization between Day 1, Day 11, and Day 21. See figure 3.

Whole body RER
RER was higher ($p<0.05$) on Day 0 than on Day 10 and Day 20. There was no difference between Day 10 and Day 20.

Other Physiological Variables
VO_2 max was decreased at the midpoint of the training which was at an altitude of 2683 meters versus 1006 meters for Pre and Post Testing. No statistical differences were observed in max power, body composition, Body weight, or Water Turnover. See Table 1.

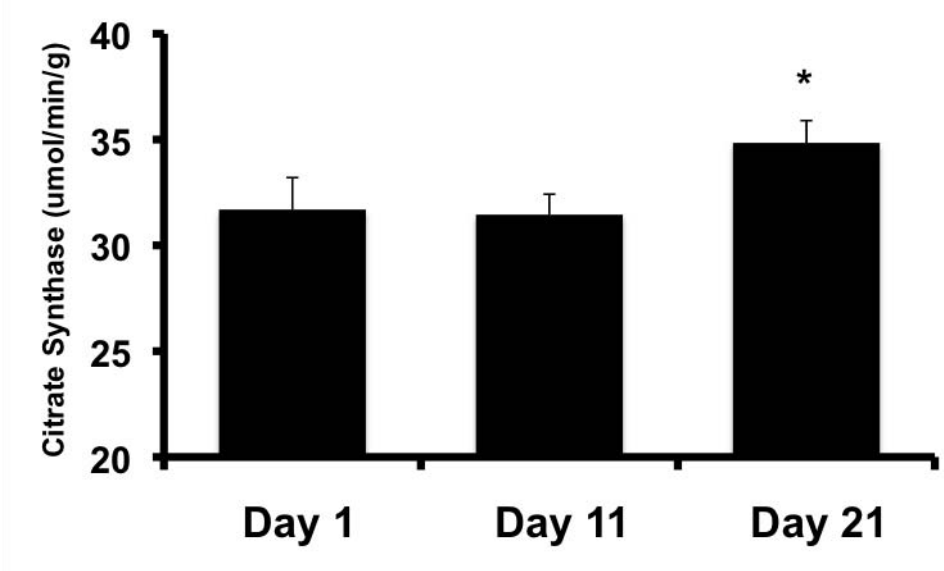

Figure 1. Maximal citrate synthase activity on day 1 (Pre), day 11 (Mid) and on day 21 (Post). *$p<0.05$ from Mid.

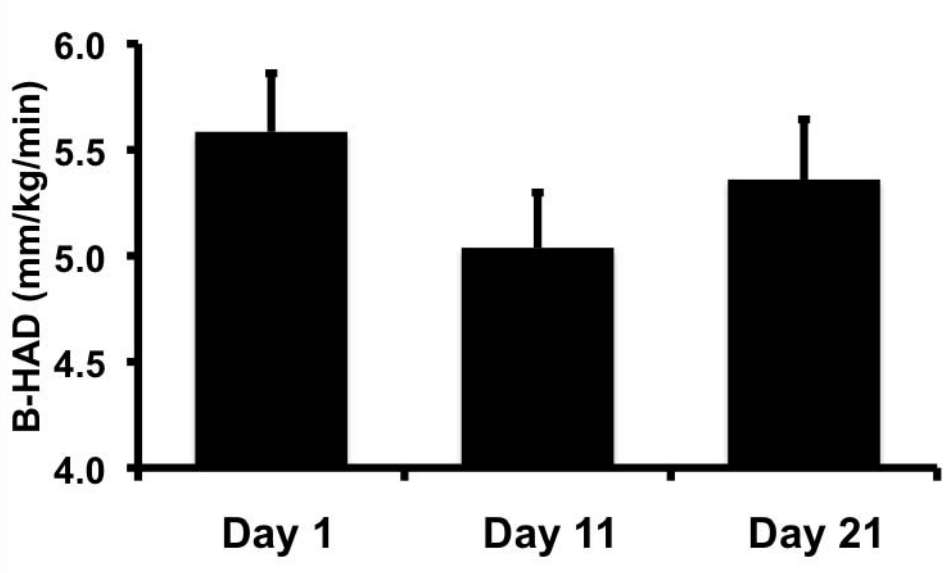

Figure 2. Maximal B-HAD activity on day 1 (Pre), day 11 (Mid) and on day 21 (Post).

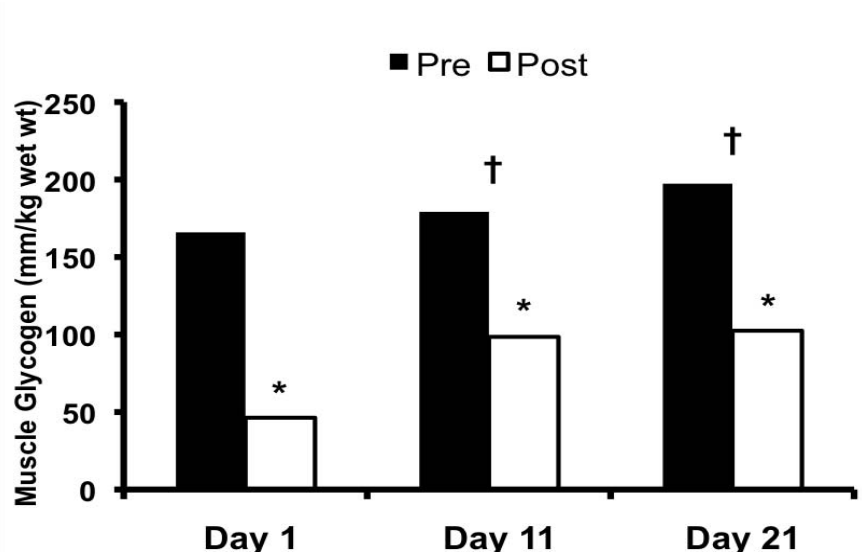

Figure 3. Muscle glycogen Pre and Post exercise on Day 1, Day 11, and Day 21 of intensified training. *p<0.05 from Pre, †p<0.05 from Day 1.

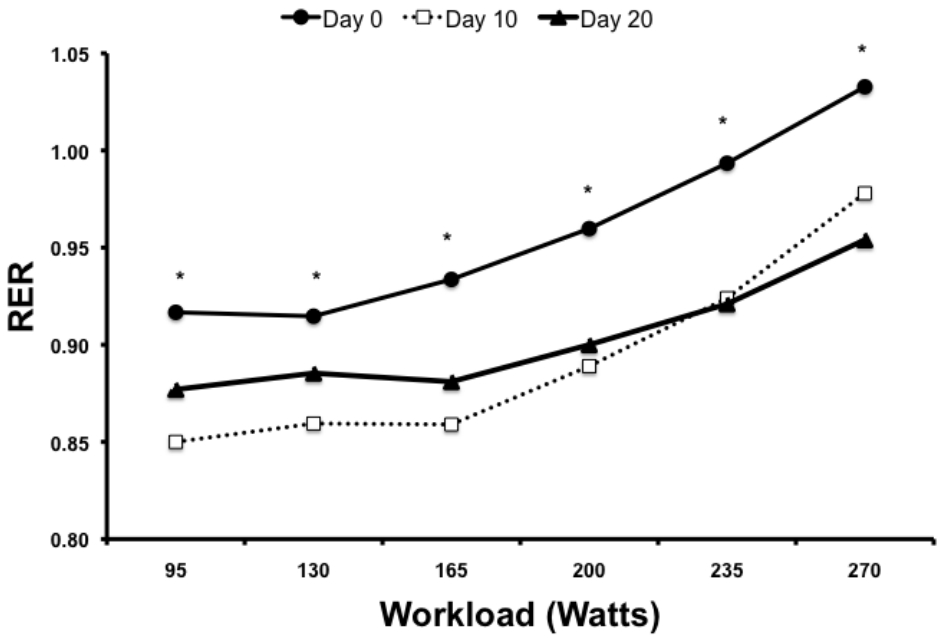

Figure 4. RER at different workloads on Day 0, Day 10, and Day 20. *p<0.05 from Day 10 and Day 20.

Table 1. Physiological measures during 21 days of intensitied training			
	Pre	**Mid**	**Post**
VO_2 max (L/min)	4.49 ± 0.11	4.08 ± 0.17*	4.57 ± 0.19†
max power (watts)	355 ± 12	341 ± 14	365 ± 14
Body Composition (% fat)	11.1 ± 1	NA	9.9 ± 0.7
Body Weight (kg)	72.2 ± 2.5	72.2 ± 2.4	71.5 ± 2.2
Water Turnover (L/100 miles)	4.2 ± 0.4	4.8 ± 0.3	4.7 ± 0.4
*p<0.05 from Pre, †p<0.05 from Mid			

Discussion:

The main findings associated with this study were that many of the intensified training induced adaptations were observed within the first ten days with no significant improvements occurring over the course of the last ten days.

The metabolic enzyme involved in beta oxidation (B-HAD) did not show any change over the 21 days of intensified training while the krebs cycle metabolic enzyme citrate synthase did show improved maximum activity from day 11 to day 21. The lack of improvement from pre to post intensified training in the metabolic enzymes may be due to the initial fitness of the subjects or the time course needed for these adaptations.

Whole body substrate oxidation and local (vastus lateralis) substrate stores did change within the first 10 days of training (even though there was no change in B-HAD or CS during this time) with no further changes occurring during the additional ten days. Muscle glycogen stores were increased. Since muscle glycogen is a preferred fuel during exercise the increased availability may allow the individuals to exercise longer at a high intensity. RER was decreased at each workload. RER reflects the whole body substrate use patterns. When RER is decreased at a given workload (as it was in this study) more fat is oxidized and less carbohydrate, thus sparing carbohydrate to be used later in exercise when in may have otherwise been used. These quick adaptations in substrate use are an important physiological adaptation to improved fitness.

The classic marker of aerobic fitness is VO_2 max. VO_2 max as well as max workload was decreased at the midpoint of the training. This decrease was contributed to by the elevation at which the test was conducted. The pre and post tests were conducted at 1006 meters while the mid test was conducted at 2683 meters. The pre and post VO_2 max values were not statistically different. This was not surprising as the individuals participating in this study were trained at the onset of the intensified training.

Body weight and body composition did not change over the course of the 21 days of intensified training. The individuals in who participated in this study were lean at the beginning of the intensified training and thus did not have excess body fat. The fact that weight and body composition was maintained is an indicator of proper energy balance during the training. Participants were allowed to eat as much as they wanted from supplemental nutrition while they

training and catered breakfast and dinner.

Water turnover was not different between pre, mid, and post testing. Water turnover was calculated during the course of cycling 100 miles at each timepoint. Water turnover is a dynamic marker of hydration flux, as it reflects the amount of water that is lost and replaced. The current data indicated that when intake is constant that water turnover does not appear to be a training induced adaptation in trained individuals.

Conclusions:

The current data demonstrates that a short period of intensified training (less than 10 days) is enough to show favorable changes in metabolism. These changes will allow an individual to exercise/work longer and harder without decay of physical and mental capacities. The use of short intensified training strategies may provide useful in a periodized training program or as a pre military deployment protocol to quickly and effectively enhance human performance and thus upgrade the human weapon system.

Field Based Study 14: Effects of 21 days of intensified training on overtraining and markers of overtraining in locally competitive cyclists.

D. Slivka, W. Hailes, J. Cuddy and B. Ruby

Montana Center for Work Physiology and Exercise Metabolism
The University of Montana
Missoula, MT
59812

Introduction:

The mechanisms, criteria, symptoms, and definitions for overtraining are poorly understood and inconsistent. Overtraining has been defined as an accumulation of training and/or non-training stress resulting in long-term decrement in performance capacity with or without related physiological and psychological signs and symptoms of overtraining in which restoration of performance capacity may take from several weeks or months. Overreaching differs from overtraining only in that it results in short term decrement of performance and takes only several days to weeks to recover. With these definitions in mind, performance is the gold standard measure for overtraining and/or overreaching despite many other proposed physiological and psychological measures.

Periods of intensified training lead to an outcome that may have a positive or negative impact on performance. In order for a positive outcome training must be in balance with adequate recovery. Negative outcomes may occur if adequate recovery is not achieved. Many terms in existing research and popular press have been used to describe this phenomenon of reduced performance after intensified training. These terms include: staleness, overreaching, and overtraining among others.

Many other markers of overtraining and overreaching have been proposed to be used as diagnostic tools, despite inconsistent findings and concrete evidence. Changes in resting and exercise heart rate, daily mood state, upper respiratory tract infection (IgA), cortisol, testosterone, and testosterone: cortisol ratio have all been implicated as markers and diagnostic tools for overtraining.

Most overtraining investigations fail to quantify the workload (including frequency, duration and intensity) of an intensified training period. This is, presumably, due to lack of an accurate and practical means to measure workload on a day-to day basis. The somewhat recent invent of devices to measure cycling power output have enabled work to be quantified during training and racing.

The purpose of this investigation was to impose a period of quantifiable intensified training to determine if commonly used markers of overtraining parallel physical performance and thus are valuable diagnostic tools for overtraining.

Methods:

Subjects
Ten male participants were recruited from the local triathlon and cycling community. These individuals were recruited due to their success in local endurance racing events and were judged by the investigators of having a realistic chance of completing the protocols involved with the current investigation. The data from two individuals are not included in the current data set due to missing data and/or technical issues. Therefore the data represented in this paper are for 8 (n=8). The protocol was approved by the Institutional Review Board (Protocol #243-06).

Training, living conditions, and stresses
The period of intensified training was organized as a 21 day out and back bicycle tour. Participants cycled at a self-selected work rate for the bulk of the training (except the 1 hr performance trial, see below). A hot breakfast and dinner was provided from a catering service that specialized in bicycling tours. During time spent cycling, participants were provided with commercially available sports food bars and drinks. Nights were spent at campgrounds in tents. By providing these accommodations daily stresses, outside of the daily cycling exercise, was minimized.

Performance Trial
On days 1, 4, 7, 11, 14, 17, and 21 a one-hour performance trial was conducted. On these days riders rode 5-10 km as a warm up before starting the performance trial. Riders were started in 2-minute intervals and instructed to go as hard as they could without drafting during the next 1 hour. The average watts produced during this 1-hour performance trial were recorded as performance capacity. Distance and speed were not used as the performance measure since the terrain varied by day (except days 1 and 21 which were on the identical course).

POMS
The Profile of Mood States QuikScore forms (MHS, North Tonawanda, NY) were used to track mood state during the intensified training period. These forms allow for measurement of: total mood disturbance, tension, Depression, Anger, Vigor, Fatigue, and Confusion. This 30-question form was administered to participants in the morning before any activity on days 1, 4, 7, 11, 14, 17, and 21.

HR
Morning resting HR, sub-maximal stepping HR, and HR after 30 seconds of seated recovery were measured on days 1, 4, 7, 11, 14, 17, and 21. Resting HR was measured immediately upon waking during 5 minutes in a seated position. Participants then stepped up and down on an 8-inch step to a metronome beat of 88 beats per minute thus prompting a step rate of 22 steps per minute for one minute. Participants then immediately sat down and recovery HR was recorded at 30 seconds post-exercise. The post-exercise recovery HR was then converted to percent of resting HR recovery.

Saliva Collection
Saliva samples were collected by passive drool technique on the morning and immediately after

cycle training on days 1, 4, 7, 11, 14, 17, and 21. Salivary flow rate was calculated by recording the time each individual took to fill a collection vial to 3 ml. The samples were immediately frozen on dry ice untill they could be transferred to an -80°C freezer and stored for later analysis.

Testosterone and Cortisol
Salivary testosterone and cortisol were measured in samples that were collected in the morning and after cycle training on days 1, 4, 7, 11, 14, 17, and 21. Salivary testosterone and cortisol were measured using a competitive immunoassay on a plate reader (Model 680 XR, Bio-Rad, Hercules, CA) at 450 nm in accordance with the manufacture's protocol (Salimetrics, State College, PA).

Secretory IgA
Salivary Secretory IgA was measured on the mornings of days 1, 4, 7, 11, 14, 17, and 21 using a competitive immunoassay on a plate reader (Model 680 XR, Bio-Rad, Hercules, CA) at 450 nm in accordance with the manufacture's protocol (Salimetrics, State College, PA). Absolute secretory IgA was then corrected for salivary flow rate.

Maximal exercise capacity
Maximum oxygen consumption (VO_2max) and workload associated with VO_2max were measured for each subject using a graded exercise protocol (starting at 95 watts and increasing 35 watts every three minutes) on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). Participants rode to volitional fatigue. Maximum workload was calculated as the highest completed stage (in watts) + the proportion of time multiplied by the 35 watt stage increment. Expired gases were collected during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and recorded at 15-second intervals.

Statistics
Differences in performance and overtraining parameters over training days were compared using one-way repeated-measures ANOVAs. Salivary testosterone, cortisol, and testosterone to cortisol ratio were compared over training days and by time of day (pre vs. post acute exercise) using two-way repeated-measures ANOVA's. In the event of a significant F ratio the false detection rate method (Benjamini and Hochberg 1995) was applied (R Development Core Team 2007) to locate differences and correct for multiple comparisons. All ANOVAs were performed using SPSS for windows Version 9 (Chicago, IL). A probability of type I error less than 5% was considered significant ($p<0.05$). All data are reported as means ± SE.

Results:

Training
During 21 days of intensified training the participants cycled 3,110 km (164 ± 5 km/day) over varied terrain. The riders averaged 170 ± 4 watts. The riders spent 51.7 ± 1.8% of the riding time at an intensity less than 50% max watts, 25.8 ± 0.7 % between 50% and 70% max watts, 15.3 ± 1.0% between 70% and 90% max watts, and 6 ± 0.4% above 90% of pre training maximal power output.

Performance
Average Power output (in watts) during a one hour time trial was no different (p>0.05) on day 4 (+2±2%), day 7 (+2±2%), day 11 (-6±3%), day 14 (+5±3%), day 17 (+3±2%), or day 21 (+4±4%) than it was on day 1 (figure 1).

Heart Rate
There was no change (p>0.05) at any time point in resting HR, step test exercise HR, or percent HR recovery 30 seconds post exercise from day 1 values (figure 2).

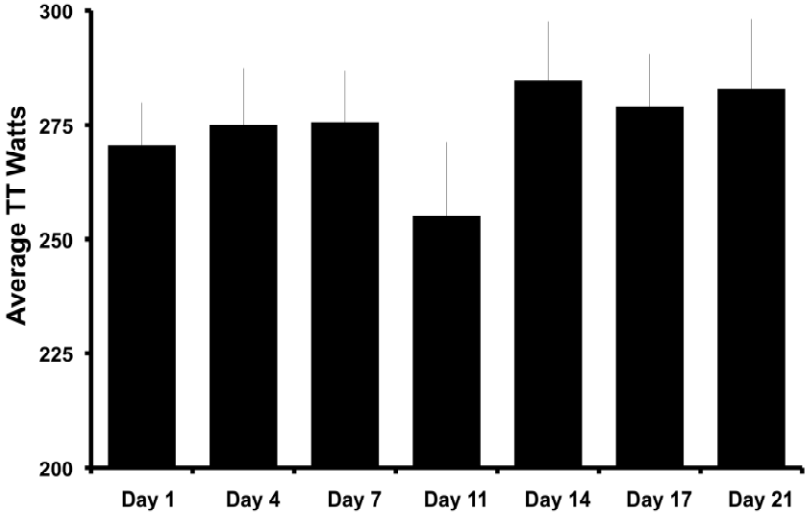

Figure 1. Average power output during a one-hour time trial over the 21-day intensified training period.

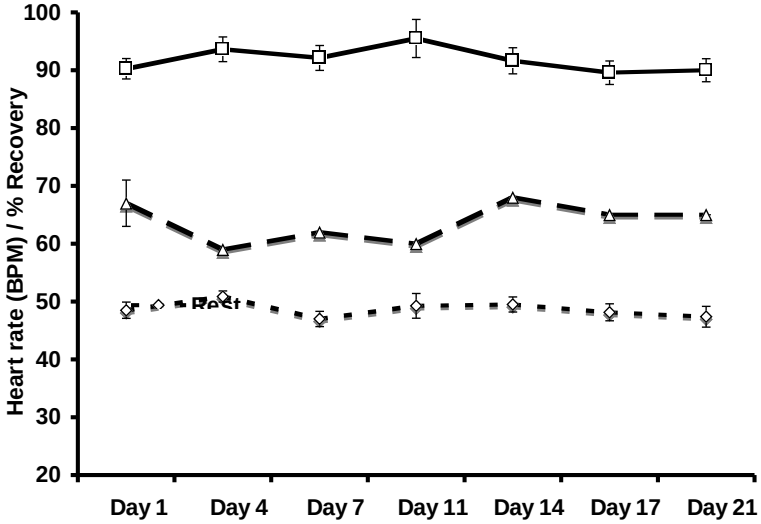

Figure 2. Morning resting heart rate, one-minute step test exercise heart rate, and % heart rate

recovery in 30 seconds during 21 days of intensified training

Immune function
There was no change in salivary IgA in absolute terms (ug/ml) or relative to salivary flow rate (ug/min, figure 3) from day 1.

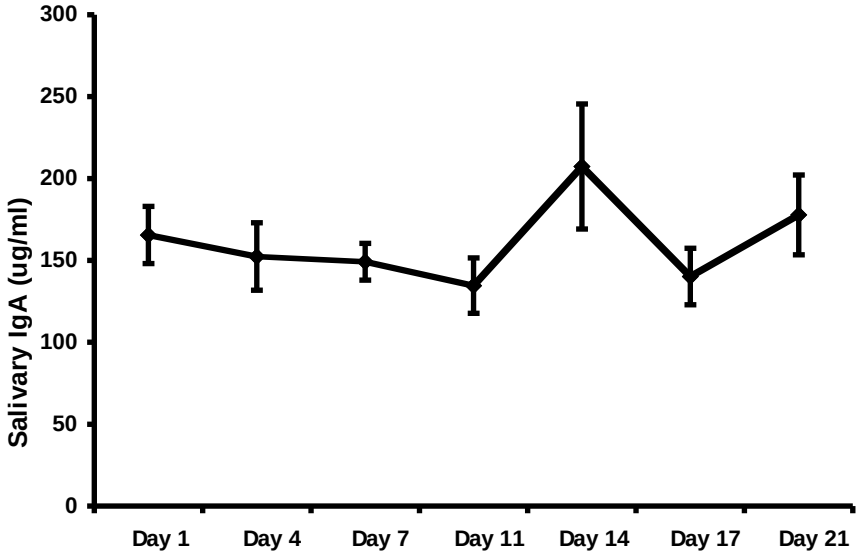

Figure 3. Morning Salivary IgA during 21 days of intensified training

Hormones

Salivary testosterone and salivary cortisol both decreased from pre daily exercise to post daily exercise ($p<0.05$) with no change over the 21 days of intensified training. The testosterone to cortisol ratio increased ($p<0.05$) from pre daily exercise to post daily exercise, but did not change over the 21 days (figure 4).

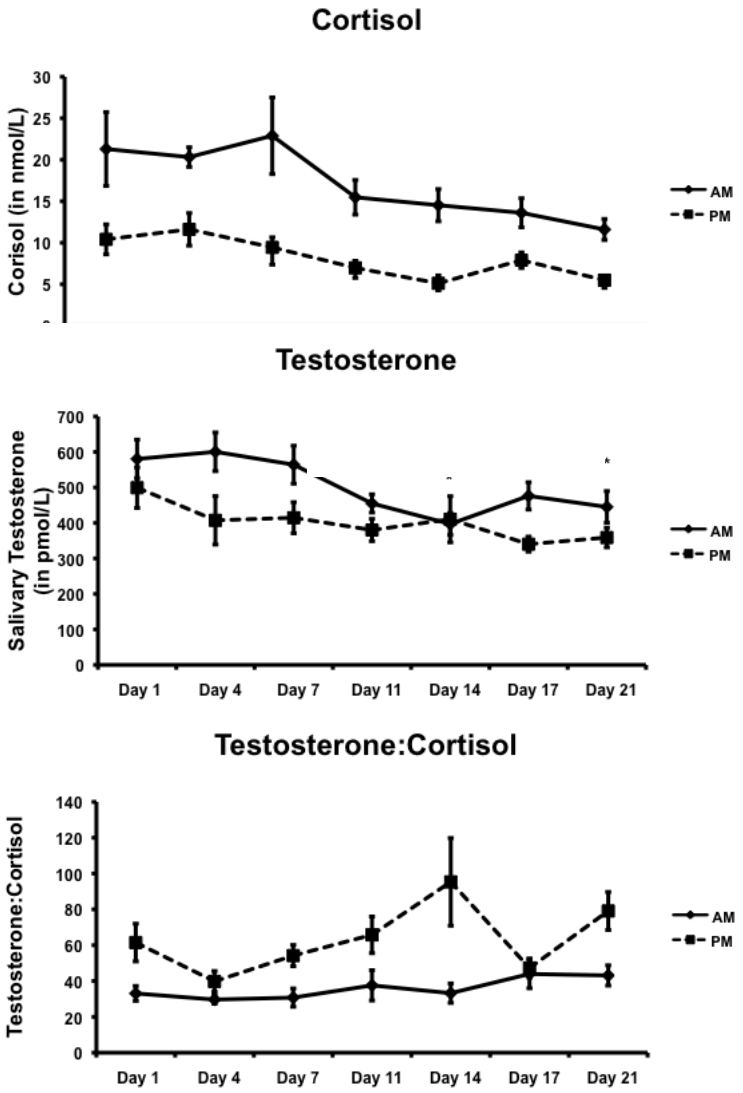

Figure 4. Testosterone, Cortisol, and Testosterone: Cortisol ratio response to daily and 21 days of intensified training.

Maximum exercise capacity

Maximum exercise capacity did not change over the course of 21 days of intensified training in terms of peak oxygen consumption (4.49 ± 0.11 $L \cdot min^{-1}$ pre, 4.57 ± 0.23 $L \cdot min^{-1}$ post, $p>0.05$) and peak workload (355 ± 14 watts pre, 365 ± 17 watts post, $p>0.05$).

Profile of Mood State

There was no change in total mood disturbance, Tension, Depression, Anger, Fatigue, or confusion over 21 days of intensified training. Vigor decreased from day 1 to day 4 ($p<0.05$) and remained lower throughout the rest of the 21-day training cycle (figure 5).

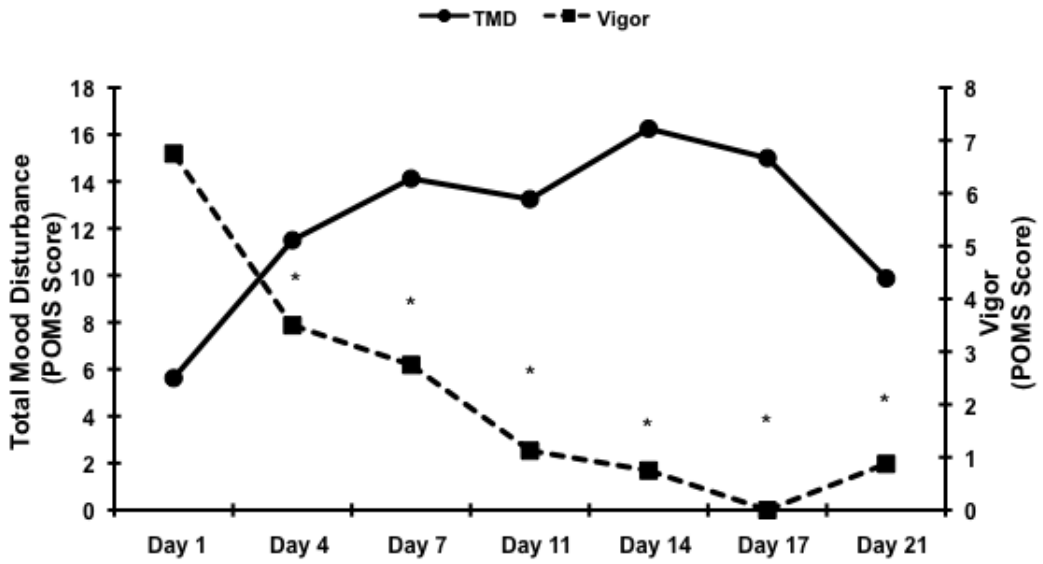

Figure 5. Total mood disturbance and vigor scores from the profile of mood states questionnaire throughout the 21 day intensified training period.

Discussion:

During 21 days of intensified exercise training there was no decline in performance and only minimal changes (POMS; vigor) in previously proposed markers of overtraining. By definition, the participants in this study did not reach a state of overreaching or overtraining since there was no decline in performance. The ~300% increase in training volume observed during this period was hypothesized to stimulate a state of overreaching and overtraining. Previous investigations that have investigated periods of intensified training, with a much smaller increase in training volume, have noted performance decline. It is not obvious to the authors why the participants in the current study responded in a favorable manner. The minimization of daily stress outside of exercise training may have allowed for an environment that was favorable for periods of intensified training. Optimal nutrition was provided and daily living stresses were controlled through the bicycle tour format incorporated into this investigation. By removing these stresses, more energy can be dedicated to dealing with the stress exerted by the exercise training. Additionally, the current participants were trained exercising individuals. While the protocol induced a large increase in training volume, all of the subjects were locally competitive athletes.

Conclusions:

The current data indicates that extreme periods of intensified training can be implemented in a safe manner without negative consequences in trained individuals. The key to success may be limiting other life stresses.